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SPECIAL LISTING

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CURRENT CANCER RESEARCH

on

SELECTED ASPECTS OF CANCER-RELATED NUCLEIC ACID BIOCHEMISTRY

OCTOBER 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service ● National Institutes of Health ●
National Cancer Institute

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CURRENT CANCER RESEARCH

I. DNA BIOCHEMISTRY

A. ISOLATION AND CHARACTERIZATION OF NUCLEAR AND MITOCHONDRIAL DNA

1. HLA REGION GENES - ANALYSIS OF NUCLEIC ACIDS

Bodmer, W. F., Lee, J. S., Imperial Cancer Research Fund, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

The main objective of my postdoctoral work is to study the regulation of HLA region gene expression in various human cells by comparing the structures of the DNAs in different tissue types and from different individuals. In order to accomplish this goal, I hope to derive analytical probes for the HLA region genes. Two different approaches will be followed. First, I will attempt to isolate a fraction of RNA enriched for HLA mRNA, transcribe the RNA into DNA, and clone the DNA in bacteria. The second approach will be to isolate DNA from an already defined human-mouse somatic cell hybrid line containing only a very small amount of human DNA which includes the HLA region. This DNA will be hybridized to RNA enriched for the HLA mRNAs. The RNA-DNA hybrids will be purified, and the DNA will be amplified in bacteria. The HLA containing clones will be identified by hybridization on nitrocellulose filters or by antibody radioassays, but final confirmation of HLA specific DNA in clones will rely on DNA sequence analysis and comparison of known amino acid sequences for the HLA antigens. After cloned DNA has been obtained, it will be mapped by cleavage with restriction endonucleases, as has been done for many cell and viral DNAs. The same methods can be used in conjunction with DNA hybridization techniques to map the sequences within the DNA isolated from various genotypes of human cells.

2. DETERMINATION OF THE DNA SEQUENCES OF THE HLA-A2 LOCUS

Pereira, D. A., Yale University, School of Medicine, Dept. of Human Genetics, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

I propose to isolate and determine the primary structure of the DNA sequence encoding the HLA-A2 haplotype. This objective will be pursued through the steps listed below. First, two oligonucleotides of 11 and 9 nucleotides, corresponding respectively to the amino acid residues 7-10 and 58-61 of the HLA-A2 peptide, will be synthesized in vitro. In the second step the membrane-associated polysome of the lymphoblastoid cell line, RPMI 4265, will be isolated and their mRNA extracted on a oligothymidylate (oligo(dT)-cellulose column. Third, those mRNA species corresponding to HLA-A2 will be identified and a complementary DNA (cDNA) fragment synthesized by sequentially annealing (to the total mRNA) and extending the 11-mer and 9-mer synthetic probes by reverse transcriptase. Finally, the cDNa will be made double-stranded (ds) and cloned, using the X1776-pBR322-EK2 host-vector system for amplification of the the HLA-A-DNA fragment. The amplified fragment will be used both as a source of DNA for further sequencing studies and as a probe through which native HLA-A-DNAs of embryonic and cultured cell line arisen will be isolated and sequenced for comparison to the biologically active mRNA.

3. GENOME ORGANIZATION OF MURINE MAJOR HISTOCOMPATIBILITY COMPLEX

Singer, D., Sachs, D. H., Abelson, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

This study was initiated to determine the DNA sequence organization of the genes encoding the murine major histocompatibility complex and the mechanisms controlling the expression of these genes.

4. DNA SEQUENCE RECOGNITION BY PROTEINS

Smith, H. O., Johns Hopkins University, School of Medicine, Dept. of Microbiology, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

The objective is to use restriction and modification enzymes as model systems for study of protein-DNA interactions. The HhaII r and m genes from Haemophilus haemolyticus have been cloned in E. coli. They are contained on a 2.2 kb DNA fragment inserted into pBR322. We have just completed the sequencing of the genes and are in a position to further engineer the recombinant plasmid to obtain higher enzyme yields. The plan is to put the genes under lac promotor-operator control and control the expression through a repressor (iQ) plasmid. This will avoid loss of the recombinant plasmid due to expression of the R-M system (a problem that has led to accumulation of r- mutants and low enzyme yields). Once a stable clone is obtained we will produce large quantities of enzymes for biochemical, physical studies, and X-ray crystallography of the protein structure.

5. PROTEIN-DNA INTERACTIONS IN HUMAN CHROMOSOMES

Latt, S. A., Kunkel, L., Lalande, M., Childrens Hospital Medical Center, *300 Longwood Ave., Boston, Massachusetts,* 02115, United States of America

The purpose of the proposed research is to investigate, at a biochemical and cytological level, the structural organization of human chromosomes and to determine the interrelationship of chromosome structure, the control of chromosome replication and X-inactivation, and sister chromatid exchange (SCE) formation. Fluorescent techniques involving pairs of dyes, many of which can serve as energy donor-acceptor pairs, will be used to elucidate chromosome structure and to highlight the staining of specific chromosomal regions. Many of the biochemical experiments proposed will explore aspects of chromosome replication kinetics and SCE formation characterized in cytological material using BrdU-dye techniques. Emphasis will be placed on a analysis of the chemistry of the SCE process and the significance of SCEs in DNA repair, the response of cells to mutagens-carcinogens, and the increased susceptibility to neoplasia in recessively inherited human chromosome fragility diseases. Biochemical and cell biological techniques, some of which involve fluorescent activated cell and chromosome sorting, will be developed and coordinated to study chromosome structure and function.

BIBLIOGRAPHIC REFERENCES: Latt, S.A., Juergens, L.A., Matthews, D.J., Gustashaw, K.M., and Sahar, E.: Energy transfer-enhanced chromosome banding (an Overview). Cancer Genet. Cytogenet. 1:187-196 (1980). Latt, S.A., and Schreck, R.R.: Sister chromatid exchange analysis. Am. J. Hum. Genet. 32:297-313 (1980).

6. DNA SYNTHESIS AND REPAIR STUDIES ON INITIATION SITES AND GROWTH OF REPLICONS IN RELATION TO CHROMATIN STRUCTURES

Ockey, C. H., Paterson Lab., *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVE: To establish a quantitative approach to the behavior of initiation sites, rates of DNA chain elongation and

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the repair of DNA under different conditions of growth of cancer-prone conditions and the response to various treatments of mammalian cells in culture using DNA autoradiographic procedures.

APPROACH: Mammalian cells in culture are pulse-chase labelled for various periods with 3H thymidine and 3H deoxycytidine. Extracted DNA is autoradiographed and measurements taken of the various parameters. The effect of drug or irradiation treatments and the behavior of DNA synthesis in cancer-prone conditions (ataxia, Bloom's and basal cell naevus syndromes) have been investigated.

PROGRESS: A relationship has been established between distance between initiation sites (ID) and rates of chain growth (R) in mammalian DNA which can be expressed under certain labelling protocol conditions in the form of a mean linear regression R: equals delta plus (K ID) 10 to the minus second power.

Changes in labelling protocol alter the values of K, ID and delta. In Bloom's syndrome, rates of chain growth in DNA are low only when cells are grown at low density, normal rates of chain growth are obtained at high density growth or in richer medium or after 3 days at low density. DNA repair after UV irradiation or methyl-methane sulphonate treatment can be visualized successfully by DNA autoradiography.

7. DNA STRUCTURES WHICH STIMULATE GENETIC RECOMBINATION AND DNA REPAIR

Hays, J. B., University of Maryland, Baltimore County Campus, Graduate School, Dept. of Chemistry, *Catonsville, Maryland,* 21228, United States of America

The nature of DNA structures which stimulate genetic recombination, of the proteins which act on them, and of the biochemical mechanisms of this action are to be studied.

Recombination stimulated by DNA repair, principally UV damage, will be studied in Escherichia coli, mostly using lambda phase systems. Objects of interest include the nature of the recombinogenic photoproduct, required E. coli gene products (for recombination in the absence of DNA replication), and the roles of the Rec A protein and of DNA gyrase. Principal approaches: in vivo studies (repressed infections) using UV-irradiated lambda phages and appropriate E. coli mutants; a cell-free system for UV-stimulated recombination; packaging of damaged DNA into lambda virions in vitro and analysis of subsequent infections.

The arl mutants, novel hyper-rec strains of E. coli identified in our laboratory will be further characterized. Principal goals: chemical structures of the 'Arl-' lesions produced in phage and plasmid DNA by these mutants; nature of the arl gene product; mechanism of stimulation of recombination by 'Arl' lesions; mode of accumulation of the (nonheritable) lesions.

New hyper-rec mutants will be identified using four new screening techniques.

Assays and techniques developed in work described above will be used for initial studies of recombination in eukaryotic cells.

8. STRUCTURE OF THE GENE FOR G3PDH IN MAMMALIAN CELLS AND REGULATION OF ITS EXPRESSION IN NORMAL AND TRANSFORMED CELLS (ABBREV)

Jeanteur, P., Blanchard, J. M., Cathela, G., Milhaud, P., Piechaczyk, M., Universite de Montpellier II - Univ. Scis et Tech. du Lang., Laboratoire de Biologie Moleculaire, *Pl. Eugene Bataillon, 34060 Montpellier, France*

OBJECTIVE: To seek answers to the following questions: What is the structure of the gene for glyceraldehyde 3-phosphate dehydrogenase (G3PDH)? Are there intervening sequences? Production of G3PDH seems to have escaped normal control in transformed cells. At which level does this control operate?

APPROACH: 1) cDNA and genomic DNA probes will be prepared by molecular cloning. 2) Comparison of these cloned DNAs will answer the question of whether the gene is split or not. 3) Using these clones as probes, we will quantitate G3PDH specific sequences in hnRNA and free and polysomal mRNA.

PROGRESS: We are currently in the final stage of positive identification of mouse cDNA clones.

9. MOLECULAR ANALYSIS OF A EUKARYOTIC GENE REGARDING DNA AND CHROMATIN STRUCTURE

Daneholt, B., Andersson, K., Wieslander, L., Karolinska Institutet, Dept. of Medical Cell Genetics, *Solnavagen 1, Box 60400, S10401 Stockholm 60, Sweden*

OBJECTIVE: In order to better understand the gene activation process in eukaryotic cells we wish to study the 75 S RNA gene in Chironomus tentans in both its active and inactive form, and to follow the conformational changes upon induction and repression of the transcriptive activity (the organization and structure of the chromatin fiber). In its inactive form the gene is part of a specific, complex chromosome structure, the Balbiani ring 2 chromomere, and we plan to investigate the relation between the 75 S RNA gene and this superstructure (whether there is one or more 75 S RNA genes, the location and conformation of the 75 S RNA gene within the chromomere). It will also be important to elucidate the significance of the rest of the BR 2 chromomere DNA to understand the function of the 75 S RNA gene (DNA with regulatory or structural roles) and to evaluate whether the chromomere is a functional unit (the presence of genes other than the 75 S RNA gene within the chromomere). Finally, we want to isolate the non-histone proteins important for chromosome structure, and we intend to initially identify and characterize the so-called scaffold proteins in the polytene chromosomes.

APPROACH: 1. Electron-microscopic analysis of changes in the structure of the 75 S RNA gene upon induction and repression of gene activity. 2. Localization of specific proteins at the 75 S RNA gene by immuno-electron microscopy techniques. Analysis of the 75 S RNA gene and the rest of the BR 2 chromomere DNA by hybrid-DNA technology: A. Preparation of a C. tentans DNA library. B. Identification of clones corresponding to the 75 S RNA gene. C. Identification of clones corresponding to the rest of the BR 2 chromomere DNA. D. Analysis of the BR 2-clones. 4. Identification and characterization of chromosomal structural proteins by gel electrophoresis.

10. DNA STRUCTURE AND GENE REGULATION

Wells, R. D., Zacharias, W., Singleton, C., Kilpatrick, M., Klein, R. D., Goodman, T., Lambert, P., Stirdivant, S., Chapman, K., University of Wisconsin, Madison Campus, School of Agriculture & Life Sciences, Dept. of Biochemistry, *Agriculture Hall, Madison, Wisconsin,* 53706, United States of America

The mechanism of gene regulation at the molecular level is being studied with special emphasis on the role of DNA properties as a determinant of specificity. One objective of this project is to identify and characterize the dynamic structure of procaryotic double-stranded DNA's, with the goal of determining the correlation between DNA physical and biological determinations will be performed on DNA's with established sequences, including procaryotic viral DNA's, highly purified DNA restriction fragments containing known genetic loci, mutant DNA's with known phenotypes and sequence changes, and DNA model polymers. The DNA restriction fragments will be purified by high pressure RPC-5 column chromatography and/or by gene cloning. Studies will be concentrated on regulatory sites containing operators and promoters with established nucleotide sequences.

An understanding of the details of gene expression is fundamental to our ultimate comprehension of normal cellular growth and division, as well as disease syndromes characterized by unregulated growth, such as cancer.

11. PRIMARY STRUCTURE OF MAMMALIAN SATELLITE DNA'S

Spencer, J. H., Ringuette, M., Queen's University, Dept. of Biochemistry, *King St. W., Kingston, Ontario,* K7L 3N6, Canada

OBJECTIVE: To obtain sequence data on cloned fragments of mouse satellite DNA and guinea pig satellite DNA's I, II and III, and to investigate the organization of the cloned DNA's in the chromsome and their chromsomal location vis-a-vis the centromere region.

APPROACH: Restriction enzyme fragments of Msp I, Eco R1, Sau 3A, Mbo 1 and Bgl II will be cloned into the M13mp7 and pUC 4 vector systems and investigated for sequence characteristics. The cloned DNA's will be used as hybridization probes to determine the localization of the restriction fragments on the

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mouse and guinea pig chromsomes. Particular attention will be paid to those cloned fragments which hybridize to the centromeric regions of the chromsomes.

PROGRESS: We have identified the repeat sequences in mouse L-cell satellite DNA in guinea pig DNA's I, II and II and the distribution of 5'-methylcytosine within the four satellite DNA's and the repetitive sequences.

12. STRUCTURE AND FUNCTION OF E. COLI POLA GENE

Grindley, N. D., Kelley, W. S., Clarke, C. M., University of Pittsburgh, School of Arts & Sciences, Dept. of Biological Sciences, *4200 5th Ave., Pittsburgh, Pennsylvania*, 15260, United States of America

DNA polymerase I of E. coli is an enzyme that appears to play an essential role in the cell both during replication, in the processing of Okazaki fragments, and in repair of damaged DNA. The recent cloning of its structural gene, polA, into the genome of bacteriophage lambda (W.S. Kelley, K.Chalmers and N.E. Murray, 1977 Proc. Nat. Acad. Sci., 74, 5632) has made possible a detailed analysis of DNA polymerase I by facilitating structural studies both of the polA gene (and thereby the amino acid sequence of the protein) and the regions controlling its expression. The polA transcriptional promoter will be defined and characterized and methods for obtaining regulatory mutants are suggested. The lambda polA phage also provides a means for fine structure analysis of the polA gene. Some polA mutations inactivate certain enzymatic functions of DNA polymerase I while leaving others intact. We propose to use the cloned gene both to map and eventually to sequence these polA-alleles.

13. STRUCTURE OF THE GLOBIN GENES IN CHROMATIN

Croissant, R., Young, N. S., Nienhuis, A. W., Goldsmith, M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Heart, Lung & Blood Inst., Clinical Hematology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The goal of this work is to determine the structural organization of the individual globin genes in chromatin. Pancreatic DNAse I and spleen DNAse II are used as probes of the structure of the globin genses and flanking DNA sequences in nuclear chromatin. Ultimately we wish to obtain a fraction of chromatin enriched in transcribed sequences. Current work focuses on the mouse erythroleukemia (MEL) cell model. Earlier studies had shown that the globin genes in nuclei from induced and uninduced MEL cells were equally sensitive to fairly extensive digestion with DNAse I. Our current efforts are focused on attempting to define more subtle differences in chromatin structure using limited DNAse I digestion. If these techniques are successful, similar analysis of the individual globin genes in nuclei from sheep and human erythroid cells will be performed to define changes in chromatin structural domains during development as the individual genes are selectively expressed. Fusion of mouse erythroleukemia cells to human fibroblasts results in stable hybrid cells which include chromosome 11. Induction of cells leads to production of mouse and human beta mRNA but the human gamma gene is not activated. By probing the chromatin structure in the nuclei of such hybrid cells with DNAse I we have shown that the beta but not the gamma gene is in an active conformation.

BIBLIOGRAPHIC REFERENCES: A.W. Nienhuis, E.J. Benz, Jr., D.M. Miller, A. Steggler, and N.S. Young: Chromatin structure and transcription of the globin genes, in 'Current Concepts of the Structure and Function of DNA Chromatin and Chromosomes, Eds. A.S. Dion, Miami, Symposia Specialists, 1979, pp. 211-228.

14. STRUCTURE OF SEA URCHIN VARIANT HISTONE GENES

Weinberg, E. S., Kaumeyer, J. F., Johns Hopkins University, School of Arts & Sciences, Dept. of Biology, *34th & Charles Sts., Baltimore, Maryland*, 21218, United States of America

The shift in the pattern of histone synthesis which occurs at blastula stage in sea urchin development is due to a transcriptional gene switch which expresses histone genes differing substantially in sequence from those which code for the histone mRNAs of the egg and early embryo. The late genes comprise 10-20% of the histone genes of the sea urchin genome and are different from the histone genes which have been previously isolated from the sea urchin.

We will isolate and clone segments of sea urchin DNA which contain the histone genes coding for the late histone mRNAs. The histone genes will be enriched by isopycnic centrifugation and gel electrophoresis and selected from E coli transformants by colony hybridization. Using restriction site mapping, Southern gel hybridization, and DNA and RNA sequencing we will determine the identity of genes contained in the cloned fragments, and the order of the structural genes. We will determine the homology of spacer sequences in the late gene DNAs with previously cloned early gene fragments using heteroduplex mapping, Southern gels, and melting curves of labeled fragments of early gene spacers hybridized to late gene DNA.

15. STRUCTURAL ANALYSIS OF ARGININE GENE REGULATION USING WILD AND SYNTHETICALLY ALTERED GENES

James, E., University of Kentucky, School of Medicine, Dept. of Biochemistry, *Limestone & Euclid, Lexington, Kentucky*, 40506, United States of America

In addition to determining the total nucleic acid sequence of the argF, argI and argECBH genes, this program will define the nucleotide sequence of regulatory regions including promoter, operator and the region of these genes which codes for ribosome binding sites. This work will also ascertain the nucleotide-protein contact points in these control regions. An important aspect of the proposal involves the construction in vitro of defined point, deletion, substitution and duplication alterations in the argF control region. This study will be complemented by the isolation and biochemical analysis of mutations in the argF control region. We have already shown that the argF gene has a leader sequence. It is proposed to determine whether a leader polypeptide is formed. The expression of the argF gene will be studied by in vitro transcription-translation under the influence of holorepressor, rho factor, ppGpp, and other potential effectors, determining the level of specific mRNA synthesis corresponding to the leader, early, mid and late regions of the gene together with the simultaneous measurement of extent of translation. This approach allows the identification of factors important to the control of transcription of anabolic processes within the context of overall cellular metabolism.

These objectives will be accomplished by the use of restriction analysis, nucleic acid sequencing using the Maxam and Gilbert and also the Sanger dideoxy techniques, coupled cell-free transcription-translation of selected DNA templates formed by in vitro recombination, molecular cloning and subsequent dissection of pure gene DNA fragments by restriction analysis. Analysis of regulatory control will be performed by quantitatively determining the level of mRNA sequences corresponding to defined regions of the argF and argI genes, as well as monitoring the quantity of OTCase formed under a variety of experimental conditions.

16. DNA SEQUENCE AND COMPUTER ANALYSIS OF A TUMOR VIRUS

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Improvements in the technology available for the determination of nucleic acid sequences have made it feasible to sequence the entire adenovirus type 2 (Ad2) genome. However, the quantity of sequence data and the speed at which this and allied information accumulates makes it no longer practical to assemble or analyze this information without computer assistance. Programs are being developed to assist with both of these functions. The programs developed have been and will be of general application. The areas of development will focus on the ability of the computer to recognize patterns and to analyze divergent sources of experimental data in order to generate hypotheses which can be tested experimentally.

17. DNA SEQUENCE REARRANGEMENTS DURING DEVELOPMENT

Kuner, J. M., University of California, San Francisco Campus, School of Medicine, Dept. of Biochemistry & Biophysics, *551*

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Determination events during development must be both stable and heritable over many cell generations. DNA sequence rearrangements provide a molecular mechanism with such properties. As a postdoctoral fellow in Dr. O'Farrell's laboratory, I will be testing the hypothesis that such rearrangements occur in association with determination events.

The multigene bithorax complex in Drosophila appears, by genetic analysis, to control determination events governing differentiation patterns in different body segments. If DNA from this region were available in an easily manipulable form (clone fragments), it might be possible to elucidate the molecular nature of cell determination.

Research plans include the development of new methods for cloning bithorax and other DNA regions defined by purely genetic criteria. Using modern recombinant DNA technology, further experiments are planned to determine if sequence rearrangements occur in bithorax during different stages of development.

18. ORGANIZATION OF REPEATED DNA SEQUENCES IN AFRICAN GREEN MONKEYS

Singer, M. F., McCutchan, T., Thayer, R., Hsu, H., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Nucleic Acid Ezymology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Monomeric and Dimeric units of the tandem repeats of the highly reiterated DNA of the African green monkey (C. aethiops) called alpha-component have been cloned in an E. coli plasmid system. The primary nucleotide structure of several such units has been determined. No two repeat units that have been sequenced are identical indicating that alpha-component is made up of a large number of slightly different but closely related sequences. In addition, fragments of the monkey genome of about 17,000 base pairs in length have been cloned in a lambda system. The 'library' was screened for segments containing alpha-component. Analysis of a selected group of the recombinant bacteriophage by restriction endonuclease digestion and hybridization reveals that no two are alike. Some of the cloned segments are largely repeats of alpha-component monomer. Other clones have stretches of non alpha-component sequence attached to the highly repeated sequence.

BIBLIOGRAPHIC REFERENCES: Singer, D.S.: Arrangement of a highly repeated DNA sequence in the genome and chromatin of the African green monkey. J. Biol. Chem. 254: 5506-5514, 1979. Singer, D.S. and Donehower, L.: Highly repeated DNA of the baboon: Organization of sequences homologous to highly repeated DNA of the African green monkey. J. Mol. Biol. 134: 835-842, 1979.

19. SEQUENCE AND EXPRESSION OF THE VITELLOGENIN GENES IN XENOPUS LAEVIS

Wahli, W., Dawid, I. B., Rebbert, M. L., Ryffel, G. U., Yaggi, R. B., Weber, R., Wood, B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Developmental Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

In Xenopus laevis, vitellogenin which is synthesized in the liver under control of estrogen is encoded in a small family of genes. Two of the vitellogenin genes together with long stretches of their flanking regions have been isolated from a Xenopus gene library. The structural organization of these two genes has been determined by electron microscopy. In both genes, the mRNA coding sequence is interrupted 33 times by sequences (introns) not present in mature vitellogenin mRNA.

BIBLIOGRAPHIC REFERENCES: Wahli, W. and Dawid, I.B.: Isolation of two closely related vitellogenin genes including their flanking regions from a Xenopus laevis gene library. Proc. Natl. Acad. Sci. U.S.A. 77: 1437-1441, 1980. Felber, B.K., Maurhofer, S., Jaggi, R.B., Wyler, T., Wahli, W., Ryffel, G.U. and Weber, R.: Isolation and in vitro translation of four related vitellogenin mRNAs of estrogen stimulated Xenopus laevis. Eur. J. Biochem. 105: 17-24, 1980.

20. IN SITU ORGANIZATION OF CHROMOSOMAL COMPONENTS

Bustin, M., Tahourdin, C. S., Kurth, P. D., Einck, L., Feldmann, R. J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Protein Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Antibodies specific to histones, non-histone chromosomal proteins HMG-1, HMG-2 and HMG-17 and to nucleosomes have been used to study the structure-function relation of defined chromosomal components in chromatin and chromosomes. A sensitive solid-phase radioimmune assay for chromosomal components has been developed. The interaction of IgG molecules with the chromatin subunit has been studied by the interactive computer graphics technique. The antibody molecule cannot interpenetrate between adjacent DNA strands to reach determinants in the histone regions covered by DNA. The complexing of chromosomal proteins into the nucleosomal conformation brings about conformational changes or steric hindrance so that part of the antigenic sites are masked. Nucleosomes elicit antibodies directed against determinants which are nucleoproteins composed of DNA and the N-terminal segments of the histones. Specific antibodies have been microinjected into oocytes of Pleurodeles waltlii. Antibodies to histones bring about a specific retraction of the transcriptional loops in the lampbrush chromosomes present in these oocytes.

BIBLIOGRAPHIC REFERENCES: Romani, M., Vidali, G., Tahourdin, C. S. M., and Bustin, M: Solid-phase radioimmunoassay for chromosomal components. J. Biol. Chem. 255: 468, 1980. Goldblatt, D. and Bustin, M.: Antigenicity of histones in various chromatins. Biochem. Biophys. Acta 606: 304, 1980.

21. GENOME MODIFICATION IN TUMOR CELLS

Smellie, R. M., Adams, R. L., Burdon, R. H., University of Glasgow, Dept. of Biochemistry, *Glasgow G12 8QQ, Scotland, United Kingdom*

OBJECTIVE: To study sequence arrangement of the genome of normal and tumor cells.

APPROACH: The levels and location of 5-methylcytosine in different classes of DNA, e.g., foldback, repeated and unique, will be examined following cell transformation.

22. DNA CONTENT ANALYSIS OF TUMOR SECTIONS

Cowell, J. K., Sheriff, M. U., Franks, L. M., Imperial Cancer Research Fund, Cellular Pathology Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

During the in vitro transformation of mouse epithelium, foci of altered cells appear several weeks after carcinogen treatment. The cells are not demonstrably tumorigenic and are considered preneoplastic. DNA quantitation of individual cells in these foci revealed consistent abnormalities in DNA content distribution which might prove to be of diagnostic value. For DNA quantitation to be effective as a diagnostic tool similar consistent changes must be shown during transformation in vivo. Such analysis is difficult and frequently normal, premalignant and early malignant cells can only be identified in tissue sections. We have therefore developed a method for measuring DNA contents in single cells in sections using a highly accurate microfluorimetric technique (ICRF Scientific Report 1978 - report 151).

Methanol-acetic acid fixed sections are treated with RNase and an aldehyde blocking agent, phenyl hydrazine, prior to hydrolysis and staining with pararosaniline. This preliminary procedure greatly reduces background staining. Using this method preliminary experiments have indicated differences between normal and tumor tissue.

23. DIFFERENTIAL DIAGNOSIS OF BORDERLINE LESIONS - MALIGNANT AND REACTIVE - USING MICROPHOTOMETRY - COMPARISON OF NUCLEAR DNA CONTENT

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OBJECTIVE: Morphological differential diagnosis of borderline lesions distingushing between malignant and reactive is often difficult in practice. Comparison of nuclear DNA contents of such lesions appears to provide a possible answer to the

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problem of discrimination between malignant and benign or reactive lesions.

APPROACH: Various kinds of lesions which are not easily classifiable as malignant or benign or reactive, such as dysplasia, mastopathia, lymphomas, papillomas or Grade 1 carcinoma of various sites, etc. are collected and examined using Olympus MMSP microphotometer. The specimens, both smear preparations and histology sections of the same cases, were tested using Feulgen reaction. Histograms of nuclear DNA content were provided and compared with those of normal DNAs contents.

PROGRESS: Data analysis continues.

24. CHROMATIN - CONFORMATION STUDIES

Fasman, G. D., Hardwicke, P., Brandeis University, School of Arts & Sciences, Dept. of Biochemistry, *415 South St., Waltham, Massachusetts*, 02154, United States of America

Research will be directed towards elucidation of the conformational consequences and biological significance of the interaction of both the histones and non-histone proteins, found in the nucleus, with DNA.

The research program to be pursued involves the isolation, biochemical characterization, immunological studies, and biophysical analysis of chromatin fragments containing varying numbers of nucleosomes from chicken erythrocytes. Careful control of ionic strength and composition, as well as protein content of the chromatin subunits, will permit a systematic study of the conformational features by various biophysical techniques, e.g., circular dichroism, nuclear magnetic resonance, polarization of fluorescence, etc.

It is anticipated that the existence and structural parameters of the higher levels of DNA organization can be elucidated by employing small oligonucleosomes capable of attaining stable compact structures corresponding to discrete levels of DNA organization. Detailed studies will be carried out on chromatin to study the effect of acetylation of H3 and H4 on the conformation and stability of both monomers and high molecular weight material. The effect on condensation of phosphorylated Hl will be also studied. Determination of the structural characteristics required for complete condensation of DNA, thus rendering it less accessible to enzymes, may aid understanding of the process of decondensation and gene activation.

25. ASSEMBLY OF CHROMATIN IN VITRO

Wiegand, R., Stanford University, School of Medicine, Dept. of Biochemistry, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

The DNA of eucaryotic organisms is organized into repeating nucleoprotein subunits. Each subunit is composed of a histone octamer and about 200 base pairs of DNA. These subunits, or nucleosomes, can be characterized in detail. Chromatin can be reconstituted by prolonged dialysis of histones and DNA from high salt and urea. This uncatalyzed reconstitution is unlikely to be the basis of assembly in vivo. Rapid assembly of chromatin under physiological conditions has been demonstrated by Dr. Brutlag, using extracts from Drosophila embryos, and by others using extracts from Xenopus oocytes. We propose to characterize this catalyzed assembly in detail and to purify the components involved. The product will be examined in detail in order to detezmine whether its structure is the same as that of native chromatin. The effects on assembly of many factors, including cleavage, acetylation, or phosphorylation of the histones, and the structure or sequence of the DNA, will be analyzed. In particular, we will study the effects of nicks, gaps, or superhelicity in the DNA, and determine the smallest DNA fragment which can be incorporated into a nucleosome. The species specificity of the system for both histones and DNA will also be examined.

26. CONFORMATION OF THE ALPHA-FETGPROTEIN GENE IN CHROMATIN

Anderson, J. N., Purdue University, School of Science, Dept. of Biological Sciences, *Executive Bldg., West Lafayette, Indiana*, 47907, United States of America

Previous studies have shown that micrococcal nuclease selectively excises genes which are in the transcriptionally active conformation in chromatin. The selectivity of this digestion process permits the fractionation of chromatin into transcriptionally active and inactive regions. In the proposed studies we will first use micrococcal nuclease as a probe for the alpha-fetoprotein gene in chromatin to determine if the loss of alpha-fetoprotein mRNA during normal liver development and the reappearance of alpha-fetoprotein mRNA during liver cancer are accompanied by corresponding alterations in the configuration of the alpha-fetoprotein gene in chromatin. Since chromosomal proteins are presumably responsible for the ordering of the DNA into template active regions, we will characterize these proteins associated with transcribed DNA in normal and malignant hepatocytes. In addition, chromatin reconstitution experiments will be employed to define the class of chromosomal proteins responsible for the generation of the alpha-fetoprotein genes into transcriptionally active conformations during normal and malignant development. These studies should therefore further our understanding of the transcriptional organization of chromatin during normal development and neoplastic transformation.

BIBLIOGRAPHIC REFERENCES: Conformation of Ovalbumin and Globin Genes in Chromatin During Differential Gene Expression, Bloom, K.S. and Anderson, J.N., J. Biol. Chem. 254:10532-10539, 1979.

27. ISOLATION AND CHARACTERIZATION OF A NEW CLASS OF DNA, THE LOW REPETITION FREQUENCY CLASS

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The overall goal of this project is to establish several fundamental parameters of a newly discovered class of DNA, the low repetition frequency (LRF) class. As indicated in the project description, very little is presently known of this class. We present an operational protocol for systematically determining whether a given sequence is of this class. The specific applications are then several: first, we wish to identify several E. coli clones carrying LRF sequences inserted into recombinant DNA vectors. Second, these cloned inserts will be used as specific probes to double-check the current knowledge of this class of DNA obtained in complex mixtures: Do these probes lead to the same conclusions concerning average reptition frequency and degree of genetic divergence? Third, the LRF-containing sequences will be mapped within the inserted DNA to provide such basic information as length and interspersion pattern. Fourth, the qualitative nature of these sequences will be examined where appropriate; for example, the presence of LRF-sequences in coding versus non-coding portions of specific gene sequences (such as ovalbumin) will be examined. In general, our approach is oriented towards delineating the basic boundaries and features of this class of DNA - the initial step in eventually integrating this class of DNA into current concepts of genomic structure and function.

28. ANALYSIS OF GENE REARRANGEMENTS DURING MACRONUCLEAR DEVELOPMENT IN OXYTRICHA

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Increasing evidence suggests that gene rearrangements play a role in the regulation of eukaryotic gene expression. The proposed research seeks to examine the nature and mechanism of eukaryotic gene rearrangements using the ciliated protozoan Oxytricha as a model system. During development of the macronucleus, which is the vegetatively functional genome in Oxytricha, specific processing events occur which result in the elimination of more than 95% of the organism's full size micronuclear genome. All of the DNA in the mature macronucleus exists as low molecular weight, achromosomal, gene size molecules. Macronuclear development, therefore, involves both a specific processing enzyme and specific nucleotide recognition signals.

I propose to isolate sequences which normally (i.e., in the complete micronuclear genome) flank the ends of macronuclear gene size molecules using recombinant DNA technology. Specifically, presently available cloned macronuclear genes, such as those coding for rRNAs, tRNAs, and the histone proteins, will be used to screen and obtain complementary sequences from a

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micronuclear DNA clone bank. In order to determine the nature of the specific processing signal at the nucleotide level, the isolated DNA pieces will be subjected to sequence analysis. Finally, the isolated sequences will be used to develop an assay for the purification and characterization of the specific processing enzyme involved in macronuclear development.

29. GENOME ORGANIZATION IN PROTOZOA

Gall, J. G., Yale University, Graduate School, Dept. of Biology, *206 Elm, New Haven, Connecticut*, 06520, United States of America

The major aim of the proposed study is an investigation of genetic organization in the macro- and micronuclei of the ciliated protozoa Tetrahymena and Paramecium, with special emphasis on sequence changes that occur during formation of the macronucleus. Effort will be concentrated on the genes coding for ribosomal RNA (rDNA), the histone genes, and some randomly selected cloned sequences. An examination of DNA from several protozoa and primitive multicellular eukaryotes will be made to determine in what groups extrachromosomal rRNA may occur in addition to those in which it has already been described.

30. STRUCTURE OF THE OVALBUMIN GENE IN CHICK DNA

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This is part of a broader project. A summary of this subproject is not available.

31. REPEATED DNA SEQUENCES IN THE CHICKEN GENOME

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Clustered repeated DNA sequences have been identified in the chicken genome. They represent a heretofore undescribed element of the eucaryotic genome. There is a scrambled order of sequences from cluster to cluster indicative of extensive rearrangement. This is not a contemporary process, but has occured through long periods of avian evolution. The clustered sequences are extensively methylated. The pattern of methylation is faithfully reproduced from cluster to cluster and is even found in the DNA of other avian species when these repeated sequences are present. Methylation and rearrangement of segments of the genome may be directly related, which has profound functional implications.

BIBLIOGRAPHIC REFERENCES: Eden, F.C., Burns, A.T.H., and Goldberger, R.F.: Complicated organization of a single repeated sequence in the chicken genome is revealed by cloning. JBC. 255: 4843-4853 (1980). Eden, F.C.: A cloned chicken DNA fragment includes two repeated DNA sequences with remarkably different genomic organization. JBC. 255: 4854-4863 (1980).

32. CHARACTERIZATION OF TRANSPOSABLE DNA FROM YEAST

Laten, H. M., University of Wisconsin, Madison Campus, Graduate School, Molecular Biology Laboratory, *500 Lincoln Dr., Madison, Wisconsin*, 53706, United States of America

Gene transposition in yeast has recently been reported. A gene coding for a suppressor tyrosine transfer RNA is capable of changing positions on the genetic map of Saccharomyces cerevisiae at high frequency. In order to determine the structural features of the DNA involved in the transposition it will be necessary to isolate that DNA. Isolation requires hybridization of radioactively labelled tyrosine tRNA to an agarose gel of endonucleolytically cleaved yeast DNA. The fragments of DNA carrying the genes encoding tyrosine tRNA define eight size classes and therefore eight unique sites in the yeast genome. If DNA from a strain characterized by transposition is cleaved, it is expected that the gel position of one of these eight will change, indicating that the gene occupies a new site in the yeast genome. Once identified and purified by the above means, it will be ligated to a specialized lambda bacteriophage and the hybrids used to infect E. coli. DNA carrying the transposed sequence can then be recovered in sufficient quality to study the physical structure of this sequence.

33. STRUCTURE OF THE YEAST MATING-TYPE LOCUS

Thorner, J., Brake, A. J., University of California, Berkeley Campus, School of Letters & Science, Dept. of Bacteriology & Immunology, *Berkeley, California*, 94720, United States of America

OBJECTIVE: To isolate DNA segments corresponding to the mating-type locus (MAT) of the yeast Saccharomyces cerevisiae from hybrid yeast-pMB9 plasmids. These DNA molecules will be used to determine the structure and organization of DNA sequences in this chromosomal region and to study the nature of the genetic transpositions involved in mating-type interconversion.

APPROACH: Clones of the hybrid plasmids will be screened for the presence of sequences derived from the yeast pgk1 gene, which maps near MAT on chromosome III of Saccharomyces. Such plasmids will then be used to search for partially overlapping segments of yeast DNA. These DNA molecules will be analyzed by restriction digestion and hybridization to provide a map of this region of the chromosome, including the mating-type locus.

34. GENETICS OF INTERVENING SEQUENCES IN YEAST

Botstein, D., Neff, N. F., Massachusetts Inst. of Technology, School of Science, Dept. of Biology, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

Interruptions in the DNA sequence which do not appear in the final mRNA have been documented for several genes from eucaryotic organisms. This noncolinearity of DNA and RNA has been found also in the simple eucaryote yeast, where the DNA sequences of tRNA genes contain intervening sequences. During the fellowship period I plan to develop a system to investigate the function of intervening sequences in yeast.

DNA sequences coding for already well-characterized yeast proteins will be isolated by screening colonies of Escherichia coli containing yeast DNA on plasmids by an immunological method. Hybridization of yeast mRNA to the isolated yeast DNA sequences will detect an intervening sequence or region of non-homology between the DNA and the RNA.

Mutants in yeast which effect the expression of the isolated gene will be selected by classical methods or in the isolated sequences in E. coli and returned to yeast by transformation. Mutants effecting RNA processing should be conditionally lethal and may effect expression of many genes in yeast.

35. BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF NUCLEIC ACIDS RELEASED BY HUMAN LYMPHOCYTES IN VITRO

Maurice, P. A., Anker, P., Hopital Cantonal, Dept. of Medicine, *54 Ave. de la Roseraie, 1211 Geneva 4, Switzerland*

OBJECTIVE: To characterize some biological properties of the nucleoprotein complex released by human blood lymphocytes and of the DNA extracted from this complex.

APPROPACH: Resting and antigen stimulated lymphocytes excrete in vitro a DNA containing complex. Furthermore, DNA released by antigen-stimulated lymphocytes was shown to code for a protein binding specifically to the stimulating antigen (Immunology 37 No. 4, 753-763, 1979). Recent experiments indicate that T-B cell cooperation during an immune reaction to certain antigen involves the transfer of information from T to B lymphocytes. Cooperation between T and B cells in an allogenic system indicates that the antibody formed in response to certain antigen is of the T cell donor allotype (Comptes rendus de l'Academie des Sciences de Paris, in press).

PROGRESS: 1) The role of the DNA purified from the complex excreted by T lymphocytes stimulated by various antigens is presently investigated for its helping and transfer ability. 2) The helper and suppressor properties of T lymphocytes separated according to the technique of Moretta et al. (L Moretta et al., J. Exp. Med. 146, 184 (1977)) in helper and suppressor populations are presently investigated.

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36. CHARACTERIZATION OF LYMPHOCYTE EXCRETED DNA

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Human peripheral blood lymphocytes stimulated in vitro with phytohemagglutinin synthesize extra copies of unique sequence element DNA having a complexity of about 10 percent of the genome, previously identified as 'excreted DNA' sequences. During log growth this DNA moves from high molecular weight form into the Hirt supernatant fraction, from which it can be released into the culture medium by treating the cells with 1 microgram/ml trypsin for five minutes at 37 degrees C. This trypsin-released DNA will be studied to determine its intracellular location prior to release, to assess its potential association with the cytoplasmic membrane, and to determine whether it is released in a particle associated with protein or lipid. Other membrane active compounds such as lectins and potential inhibitory compounds such as c-AMP will be studied to determine their effect on the release mechanism. Purified DNA will be studied by reassociation kinetics analysis to determine if the unique sequence elements in excreted DNA are associated with repetitive sequence elements. In addition, RNA-DNA reassociation experiments will determine whether excreted DNA is transcribed.

BIBLIOGRAPHIC REFERENCES: Distelhorst, C.W. and Rogers, J.C. 1979. Glucocorticoids Inhibit Trypsin-Induced DNA Release from Phytohemagglutinin-Stimulated Blood Lymphocytes. J. Immunology. 123:487-495.

37. PATHOGENETIC DETERMINANTS OF HUMAN CNS TUMORS

Manuelidis, L. M., Yale University, School of Medicine, Dept. of Pathology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

Our major aim is to understand the changes in nuclei of aneuploid CNS neoplasms. Isolation and characterization of subsets of repeated sequences in mouse and human DNA will be continued, with emphasis on chromosomal structure (studies by in situ hybridization) and DNA analysis. Ultrastructural studies of the nucleolus organizing regions in interphase nuclei will be correlated with quantitative analysis of DNA restriction profiles from normal and neuroectodermal tumor cells, using defined ribosomal DNA probes. Methods to analyze other DNA sequence subsets of interest, with respect to their three-dimensional arrangement in chromosomes are currently being investigated.

38. CHROMOSOME CHANGES ASSOCIATED WITH TRANSFORMATION OF MOUSE SALIVARY GLAND EPITHELIUM

Cowell, J. K., Wigley, C. B., Imperial Cancer Research Fund, Cellular Pathology Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

Changes in DNA content during in vitro transformation of mouse salivary gland epithelium has been described previously (ICRF Scientific Report 1978 - report 152). All tumorigenic cell lines transformed in vitro, or from tumours induced in vivo, had DNA contents in the subtetraploid range. This has been confirmed using conventional metaphase chromosome preparations and all but one line (from a carcinoma induced in vivo) have modal chromosome numbers in the 63-73 range (2n equals 40). The exception has a bimodal distribution with a sub-octaploid component. A full analysis of the chromosome complement in each cell line, using the trypsin-giemsa banding technique, is nearly complete. It is hoped that consistencies may exist between different lines and that comparisons can be made with information from a similar study of transformed mouse bladder epithelial cell lines (Report 141). It will be of particular interest to follow the development of chromosome patterns typical of these cell lines where stocks are available of the early passage, preneoplastic cells.

39. DEOXYRIBOSE CONTAINING COMPOUNDS IN NORMAL AND CANCER TISSUES

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This program's objectives are to isolate and identify deoxyribose-containing compounds other than DNA from normal and tumor tissues, to determine the metabolic sequences in which these compounds are involved and whether these sequences are the same in normal, precancerous, and cancer tissues, to isolate DNA from subcellular structures other than nuclei, to study the physical and biochemical properties of extranuclear DNA and to determine the relationship between nuclear and extranuclear DNA in carcinogenesis.

BIBLIOGRAPHIC REFERENCES: Gantt, R. and Schneider, W. C. Presence of 5-methylcytosine in rat liver mitochondrial and microsomal DNA. In: Developments in Neuroscience: Transmethylation. E. Usdin, R. T. Borchardt and C. R. Creveling (eds.). Elsevier/North Holland, Volume V, pp. 465-471, 1979.

40. DISPERSED REPEATED GENE FAMILIES IN DROSOPHILA MELANOGASTER

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A novel class of repeated genes in Drosophila melanogaster has been described in which the elements are widely dispersed throughout the genome rather than tandemly arranged. In this proposal we discuss evidence from this laboratory which suggests that the elements of these gene families are mobile within the genome and undergo significant rearrangement in both fly populations and tissue culture cell lines. We plan to study the mechanism(s) responsible for this observed movement by using the techniques of molecular cloning. We will isolate several cloned segments of Drosophila melanogaster tissue culture DNA which contain an inserted element of a gene family, and then use single copy sequences which are adjacent to the elements on these DNA segments as probes to screen a library of cloned embryonic DNA sequences. We will thereby isolate pairs of DNA segments from tissue culture and embryo cells which are identical except that the tissue culture cell DNA will have had an element of a dispersed gene family inserted. By comparing the structure of such pairs of DNA segments we will be able to distinguish several possible mechanisms for their movement within the genome. It is hoped that the further study of this new class of gene families will lead to a better understanding of their possible function and significance in such processes as mutation, evolution, and genetic regulatory mechanisms.

41. SEQUENCE ORGANIZATION IN THE GENOME OF DROSOPHILA MELANOGASTER

Dawid, I. B., Long, E. O., Rebbert, M. L., Lauth, M., Wellauer, P., Pardue, M. L., Wood, B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Developmental Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

We have shown earlier that a group or repeated sequences which are homologous to the ribosomal DNA insertion occur in the chromocentric heterochromatin of Drosophila melanogaster. Several cloned DNA fragments have been isolated which contain regions homologous to the insertion interspersed with other DNA sequences called flanking sequences. We have shown that some of the insertion-like sequences occur in tandem repeats within the chromocenter. An analysis of several flanking sequences showed that some are transcribed into heterogeneous nuclear RNA. The chromosomal location of flanking sequences is being studied in different strains and species of Drosophila.

BIBLIOGRAPHIC REFERENCES: Dawid, I.B. and Long, E.O.: Ribosomal DNA and related sequences in Drosophila melanogaster. In Alfred Benzon Symposium XIII, Munsgaard. Kopenhagen, pp. 103-114, 1978. Dawid, I.B., Lauth, M. and Wellauer, P.K.: Repetitive DNA elements related to the ribosomal insertion of Drosophila melanogaster. In Mobilization and Reassembly of Genetic Information, Miami Winter Symposium 16 (eds. Scott, W.R., et al.), Academic Press, New York, 1980, in press.

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42. GLUE PROTEIN GENES OF DROSOPHILA MELANOGASTER

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The Drosophila glue proteins represent an ideal system for studying control of gene expression in eukaryotes. The proteins are produced in large quantities by the larval salivary glands, and there are variants which have different amounts of two of the proteins (P3 and P5). P5 is especially interesting since it appears to be regulated by two separate loci.

I will try to find differences in the DNA sequences of the glue protein gene from stocks that produce different amounts of these proteins. I will characterize these DNA differences by cloning the genes in a suitable recombinant DNA vector. Finally I will try to correlate different DNA sequences with altered gene expression using forced recombination between stocks.

43. ISOLATION AND STUDY OF CHROMOSOMAL DNA FROM THE 3C2-3C7 REGION OF DROSOPHILA MELANOGASTER

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The general purpose of this project is to clone, isolate and study DNA from the 3C2-3C7 region of the X chromosome of Drosophila melanogaster.

Fragmented Drosophila DNA will be cloned using 'cosmids,' a technique which guarantees that large segments of chromosomal DNA will exist in each clone. Clones containing DNA from the 3C2-3C7 region will be isolated by using labelled DNA from copia (one family of repeated genes in Drosophila) genes as a probe. Once unique sequence DNA has been isolated from the region, this DNA will then be used as a probe to isolate further sequences in the region.

The DNA thus isolated will be studied to determine the size and continuity of complementation units in the 3C2-3C7 region, to map structural genes and their surrounding sequences within and around these complementation units, and to correlate the structure of these units, already defined geneticaly, with the fine structure of the DNA in the region.

The techniques used in isolating DNA from the 3C2-3C7 region should be applicable to the isolation of DNA from almost any chromosomal region in Drosphila.

44. DNA, CHROMATIN AND CHROMOSOMES OF DROSOPHILA NASUTOIDES

Lee, C. S., University of Texas, Austin Campus, School of Natural Sciences, Dept. of Zoology, *200 W. 21st St., Austin, Texas*, 78712, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

45. CHARACTERIZATION AND ORGANIZATION OF GENES CODING FOR DROSOPHILA CHORION PROTEINS

Kafatos, F. C., Harvard University, Cambridge Campus, School of Arts & Sciences, Dept. of Biology, *Cambridge Station, Cambridge, Massachusetts*, 02138, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

46. CHROMATIN STRUCTURE IN EUKARYOTIC CELLS (HUMAN, RATS)

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This is part of a broader project. A summary of this subproject is not available.

47. MITOCHONDRIAL DNA FROM NEOPLASTIC AND NORMAL CELLS

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The nucleotide sequence analysis of mouse mitochondrial DNA is being carried out, using both the Maxam-Gilbert chemical method and the Sanger chain termination method. For the latter purpose, mitochondrial DNA is being subcloned into the filamentous phage cloning vector M13.

The position of transfer RNA genes is being located by hybridizing labeled mitochondria tRNA to Southern transfers of mitochondrial DNa restriction digests. The sequencing of these genes will establish the genetic code used by mouse mitochondria.

In addition, attempts are underway to obtain translation in E. coli of mitochondrial proteins from cloned DNA.

BIBLIOGRAPHIC REFERENCES: P.H. Johnson, M.J. Miller, E. Wild, S.V. Kelly and L.I. Grossman (1979). Electrophoretic characterization of intracellular forms of bacteriophage phiX174 DNA. The identification of novel intermediates of altered superhelix density. J. Virol. 32, 629-639. P.H. Johnson, M.J. Miller and L.I. Grossman (1980). Electrophoresis of DNA in agarose gels. II. Effects of loading mass and electroendosmosis on electrophoretic mobilities. Analyt. Biochem. 102, 159-162.

48. CHARACTERIZATION AND REGULATION OF THE MITOCHONDRIAL GENOME

Rabinowitz, M., University of Chicago, Pritzker School of Medicine, Dept. of Medicine, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

The synthesis and assembly of functional mitochondria depend on the interaction between two separate genetic systems. Mitochondrial DNA codes for a limited number of mitochondrial components including the two rRNA subunits, 15-25 tRNAs, and probably 18-22 messenger RNAs. More than 90% of the mitochondrial protein is synthesized on cytoplasmic ribosomes from nuclear information. The coordination of nuclear and mitochondrial genomes is emphasized by the fact that the oligomeric respiratory carriers, such as cytochrome oxidase and oligomycin sensitive ATPase, are each composed of some peptides specified and synthesized by the mitochondrial genetic systems and others translated by cytoplasmic ribosomes and specified by nuclear DNA.

Our initial approach will be to isolate mRNAs for nuclear-specified cytochrome oxidase, and oligomysin-sensitive ATPase peptides. Highly labeled cDNA probes made from these mRNAs will be used to measure the nuclear transcription of mRNA coding for specific mitochondrial peptides during coordinated repression or derepression of mitochondrial respiratory function. Labeled cDNA transcribed from the same mRNAs would be used to scan existing collections of yeast nuclear-plasmic recombinant DNA clones, which may help to identify neighboring control sequences possibly involved in the coordinated transcription of mitochondrial mRNA.

49. MITOCHONDRIAL NUCLEIC ACID STRUCTURE

Clayton, D. A., LaVerne, L. S., Stanford University, School of Medicine, Dept. of Pathology, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

This proposal is concerned with the nucleotide sequence organization of mouse and human mitochondrial DNA. The entire sequence of mouse mitochondrial DNA will be determined and compared to that of human mitochondrial. We will determine the map position of ribonucleotides known to be incorporated into mitochondrial DNA and assay for any possible integration of mitochondrial DNA sequence in the nuclear genome. D-loop mitochondrial DNA will be utilized as a substrate for a variety of in vitro reactions with DNA polymerases to determine the properties necessary for elongation of D-loops. The methodology involves DNA sequencing, restriction enzyme digestion, gel electrophoresis, centrifugation and electron microscopy.

50. STRUCTURE AND SYNTHESIS OF DNA

Englund, P. T., Johns Hopkins University, School of Medicine, Dept. of Physiological Chemistry, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

We are studying the mitochondrial DNA from Crithidia fasciculata known as kinetoplast DNA. Using restriction enzyme mapping, DNA sequencing, and heteroduplex mapping, we are studying the organization of the minicircle sequences of this DNA. We are especially interested in determining the sequence of minicircle fragments which have extremely anomalous electrophoretic mobilities. These fragments may form unusual secondary structures. We are also studying the mechanism by

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which this DNA replicates. Our current view is that the minicircles do not replicate while attached to the network, but that they are dissociated from the network and then replicate as free minicircles. Subsequent to replication, they are reattached to the periphery of the network. We are studying the mechanism of replication of the individual free minicircles, and we are also attempting to isolate the enzymes responsible for removal of minicircles from the network and for reattachment of minicircles subsequent to replication.

BIBLIOGRAPHIC REFERENCES: Englund, P.T. (1978) The Replication of Kinetoplast DNA Networks, Cell 14:157-168.

51. STUDIES ON THE PURIFICATION AND FUNCTION OF EXTRANUCLEAR MELANIN DNA

Liacouras, A. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Nuclear Acids Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The long-range objective of this project is to investigate the function and properties of melanin DNA in murine melanomas. The initial aim is to purify the various DNA's from the nuclear, mitochondria and melanin granules, and characterize these with respect to melting points, density in CsCl and sedimentation coefficients. Nucleic acid hybridization studies will be used to determine the degree of interrelatedness between species, particularly with respect to the melanin DNA. The function of this latter DNA species will then be elucidated.

BIBLIOGRAPHIC REFERENCES: Liacouras, A.S., and Anderson, E.P.: Uridine-Cytidine Kinase IV. Kinetics of the competition between 5-azacytidine and the two natural substrates. Molecular Pharmacol. 15: 331-340, 1979.

B. DNA REPAIR AFTER UV OR IONIZING RADIATION

52. INTERACTION OF ULTRAVIOLET LIGHT AND CHEMICAL CARCINOGENESIS

Starkey, J., Washington State University, School of Veterinary Medicine, Dept. of Veterinary Microbiology & Pathology, *Pullman, Washington,* 99163, United States of America

OBJECTIVE: Determine the effect of the interaction of UV irradiation and other environmental carcinogens on DNA conformation and DNA repair mechanisms.

APPROACH: Treat mammalian cells in culture with various polycyclic hydrocarbon carcinogens and then with UV irradiation. Determine effect on DNA repair response, DNA conformation, and stability of DNA and DNA-protein complexes.

53. UV TRANSFORMATION AND DNA REPAIR IN HUMAN CELLS AND SKIN

Sutherland, B. M., Brookhaven National Lab., Dept. of Biology, *Upton, New York,* 11973, United States of America

We have developed a system for ultraviolet light (200 nm less than lambda less than 400 nm) transformation to anchorage-independent growth of human cells in culture. We shall characterize the transformation process wavelength dependence, influence of age and origin of cells, effect of irradiation and growth schedule, as well as the role of DNA and/or misrepair. We shall characterize the growth patterns, efficiency of anchorage-independent growth, karyotype and chromosomal banding patterns and tumorigenicity of the transformants.

We shall use alkaline agarose gel techniques to detect low levels of DNA damage and repair in small quantities of non-radioactive human fibroblasts, leukocytes and skin. We shall obtain action spectra for pyrimidine dimer formation in human skin and compare it to those for ultraviolet oncogenesis in the mouse. We shall determine rates of repair of dimers by excision and by photoreactivation in human skin.

We shall determine the relationship between UV transformation in vitro and solar oncogenesis in human skin, and the possible roles of DNA repair or misrepair in these processes.

54. EFFECT OF ULTRAVIOLET LIGHT ON CELLULAR PROCESSES

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The object is to understand the effects of ultraviolet radiation (UV) on cellular processes, the repair of damages caused by the radiation, and the mutational consequences on the repair processes. A recently discovered effect of UV is the suppression of polarity, which is a premature termination of transciption. A theory will be tested that the UV depletes the activity of the transcription termination factor rho. Polarity will be studied in two genetic systems, the genes of bacteriophages phi X174 and G4, and the genes of the gal operon. The aims are to explain the function of the rho protein in the repair of cell damage and how UV prevents transcription termination. UV can also cause termination of transcription and that property will be used to provide a functional test for promoters. The aims are to determine if the use of distant promoters rather than proximal ones in a general strategy of viruses and to determine what signals are involved in specifying the translatable part of a messenger RNA. UV induces a repair process that is highly mutagenic. A mechanism has been proposed to explain the mutagenic effect and the theory will be tested by direct determination of the base changes produced by the mutations, and that will be done by DNA sequencing.

55. CELL RESPONSE TO DNA DAMAGE BY UV AND OTHER MUTAGENS

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Our objective in this work is to study factors that contribute to human susceptibility to cutaneous malignancy. We want to know why some persons with certain heritable disorders and some who have been exposed to environmental mutagens develop more skin cancers than normal. We are concerned with the long-term and short-term biological consequences of photomediated, covalent psoralen-DNA photoadducts such as are produced by photochemotherapy (PUVA) with 8-methoxypsoralen (8-MOP) and UV-light (365 nm).

After in vitro binding by UV-A of 4,5'8 trimethylpsoralen (TMP) and 8-MOP we are comparing cutaneous cells and lymphocytes from persons in normal and disease states with respect to cell growth and survival, and repair of psoralen DNA cross-links. We are using cells from persons with dyskeratosis congenita and xeroderma pigmentosum and from their relatives. We are also measuring concentrations of psoralens in blood of our PUVA patients and correlating these values with the patients' phototoxic and therapeutic responses. We are comparing the cross-linking capabilities of several different psoralens.

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56. AN IMMUNOLOGICAL APPROACH TO STUDY DNA DAMAGE AND REPAIR BY IRRADIATION AND CHEMICAL CARCINOGENS

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Antibodies against DNA modified by benzo(a)pyrene diol-epoxide I (BP-DNA) were raised, purified and studied in several ways. These antibodies did not react with double- or single-stranded DNA or with BP-RNA or BP-protein. Such antibodies were used in initial attempts to detect repair of BP-DNA in treated cells by fluorescence assay, to detect BP residues on DNA by electron microscopy, to detect BP residues on the DNA of gently permeabilized cells. The feasibility of a monoclonal system for producing such antibodies is under investigation.

57. REPAIR OF DNA TREATED WITH GAMMA-IRRADIATION AND CHEMICAL CARCINOGENS

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Enzymatic mechanisms of DNA repair were investigated. An N-glycosylase active on 3-methyladenine was isolated from rat liver nuclei and purified 100 fold. Its K/sub m/ is 2.6 mM and its molecular weight is 24,000. The properties have been described. An N-glycosylase active on 7-methylguanine was partially purified from E. coli. A new derivative of adenine after reaction with beta-propiolactone has also been described.

58. DNA REPAIR MECHANISMS IN CARCINOGENESIS

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OBJECTIVES: To elucidate the molecular events in human cells when cellular macromolecules such as DNA are damaged by radiation or chemical agents by studying and characterizing the sequence of DNA repair events, the various modalities of repair, the genetic inhibition of repair due to mutation, and the physiological inhibition of repair due to biochemical inhibitors.

MAJOR FINDINGS: Normal human cells are able to repair ultraviolet (UV) light induced pyrimidine dimers in their DNA while cells derived from patients with the genetic disease Xeroderma pigmentosum (XP) show defective or reduced repair. The number and kinds of pyrimidine dimers formed in DNA by ultraviolet light is related to the DNA base ratio, the wavelength and the total exposure to UV. After 50 J/m2 at 254 nm the ratio of dimers (C greater than less than C, C greater than less than T, T greater than less than T) in human cellular DNA was 14:20:66 (24:38:38 in E. coli). A low dose of an FS40 sunlamp resulted in a ratio of 20:40:40. Normal human cells approximately 10 to the 6th power dimers within a 24 hour period after 254 nm or FS40 irradiation. About one million dimers were formed in cells by 10 J/m2 of 254 nm or 10 to the third power J/m2 of filtered sunlamp. At low doses nearly all of the dimers were excised. At higher doses dimer excision was inhibited.

Arabinofuranosyl cytosine (ara-C) at 20 micron m concentration in the presence of 2 mM hydroxyurea has been found to inhibit steps in DNA excision repair following UV irradiation and chemical insult. It causes gaps to accumulate in incised regions which can be observed on alkaline sucrose gradients. Whereas cells from XP patients show a decrease in strand interruptions following UV irradiation in the presence of ara-C (reflecting their lower excision repair capacity), cells from variant lines of XP show an increase in strand interruptions relative to normal human cells. In studies to elucidate the mechanism by which ara-C inhibits repair, it appears that ara-C is capable of at least a two-step inactivation process whereby an initial interaction with the DNA polymerase and/or exonuclease inhibits closure of repair gaps. This is followed by an apparent ara-C dose-dependent inhibition of the incision step. (Text Abridged.)

59. REPAIR OF X-IRRADIATED DNA IN NORMAL AND CANCER CELLS

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We propose to study the enzymatic removal from DNA of 7-methylguanine residues in which the imidazole rings have been opened. This compound, 2,6- diamino-4-hydroxy- 5-N-methylformamido- pyrimidine, is recognized by a partially purified fraction from E. coli which we have obtained. X-irradiated DNA will be tested with this fraction to determine if the enzyme recognizes similar bases and if so, the enzyme, a glycosylase, will be used to try to characterize those bases. Testing for this activity in mammalian cells will also be done. A second project involves the ability of radiosensitive and radioresistant lines of the murine lymphoma strain L-5178y to remove bases from DNA alkylated with MNU.

60. GEV HEAVY-ION AND 200 KV X-RAY EXCITATION OF DNA

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The energy deposition and dissipation processes in oriented DNA due to exposure to ionizing radiations will be investigated by luminescence spectroscopy. The emission spectra will be determined during excitation and post-excitation over a range of temperatures. Thermoluminescence of irradiated samples will be investigated, as well as low energy photon stimulation. Excitation sources will be 200 kV x-ray, GeV heavy ions, 60Co gamma-rays, and synchrotron radiation.

The response of the DNAs will be characterized by their corrected emission spectra, relative integrated intensity, polarization and lifetimes.

61. MODIFICATION OF X-RAY INDUCED DAMAGES IN PHAGE T4

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The enzymes responsible for repairing DNA damage also function in several biologically significant pathways such as DNA replication, genetic recombination, and mutation fixation; and are thus directly implicated in the oncogenic process. Not only is little known about the repair of ionizing radiation-induced damages in DNA, but the chemical nature of these damages remains to be elucidated. The proposed research would be undertaken in an effort to define the molecular mechanisms involved in the repair to X-ray-induced damages in DNA using T4 bacteriophage and its Escherichia coli host as model systems. To this end, the DNA synthetic patterns of the recombinational repair deficient T4 x and y mutants have been examined, and the existence of aberrant DNA replicative intermediates established. An attempt will be made to isolate the products of the x and y genes by a complementation assay. Further, the E. coli X-ray endonuclease will be purified, and its properties delineated. Lastly, irradiated phage DNA will be repaired in vitro by purified enzymes of known specificities, and assayed for biological activity by transformation or transfection.

62. X-RAY INDUCTION OF IMMUNOREACTIVITY IN ANIMAL CELL DNA

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Single-stranded regions of HeLa nucleosomal DNA will be identified by specific attachment of 125I labeled antinucleoside antibodies to different DNA species in agarose and polyacrylamide slab gels. The influence of x-irradiation and the formation of N-Ac-AAF guanosine adducts will be correlated with the presence of unwound DNA in nucleosome core and linker DNA.

Restriction endonuclease digests of phi X174 single-stranded viral DNA and of African green monkey component alpha DNA (172 b.p.) will first be studied, to find the best conditions for radiolabel staining of the gels. These DNA standards will be included in gels to provide references for 125I attachment.

After x-irradiation, DNA repair synthesis will be compared in nucleosomal core and linker DNA, as isolated from sucrose velocity sedimentation or gels. The sites of DNA repair synthesis will be compared with the location of unwound DNA and N-Ac-AAF guanosine adducts, determined by radioimmune assay or 3H-N-Ac-AFF radiolabel.

Immunoprecipitation of purified nucleosomal DNA by antinucleoside antibodies is an alternate means to determine single-stranded regions in DNA.

Diagnostic ultrasound induced cell nuclear immunoreactivity; other effects were previously reported - Radiology 131:177 (1979), Science 205: 1275 (1979). Recently we observed abnormalities of 3T3 cell membranes using scanning electron microscopy. Disoriented cell movement has also been observed and needs more study to learn if actin bundles or microtubules are involved. The cell labeling index (LI) in human cancer biopsies have been determined by the antinucleoside antibody method (Cancer Research 38: 1012 (1978)). The LI is compared with the 3H-thymidine labeling index in human mammary cancer biopsies and is also being used to correlate LI and clinical staging and response to therapy in human mouth cancer.

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63. STUDIES ON THE MOLECULAR MECHANISMS OF REPLICATION, REPAIR AND MUTAGENESIS OF DNA IN HUMAN CELLS

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OBJECTIVE: (a) Studies on the factors which facilitate the advancement of DNA polymerase at the growing fork in human cells; (b) Molecular factors involved in DNA repair of human cell chromosomes after UV irradiation or chemical damage.

APPROACH: (a) Isolation and characterization of proteins and enzymes which are able to specifically stimulate the DNA polymerase in vitro on model replication forks; (b) Isolation of metaphase chromosomes, damage with UV or mutagens, anaysis of capacity of repair synthesis either endogenous or after addition of factors presumably involved in repair (polymerases, endonucleases, etc.) and study of the effect of relative inhibitors.

PROGRESS: (a) We have identified 2 proteins which specifically stimulate pol on model replication forks: an SS DNA binding protein and a DNA dependent ATPase. (b) We have found isolation conditions which yield chromosomes that appear to be suitable for the studies described under Approach (b).

64. MUTAGENESIS AND REPAIR OF DNA

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The goal of the proposed research is to understand the cellular molecular mechanisms by which DNA damage is processed to give stably inherited mutations. The drug-resistance plasmid, pKM101, causes a striking enhancement of radiation and chemical mutagenesis when present in bacterial screening strains used to detect carcinogens and mutagens. Results to date have suggested that pKM101 enhances mutagenesis and increases resistance to UV killing by coding for functions involved in the hypothesized 'error-prone repair' functions and this relationship will be further explored. The genetics of the system will be characterized by the isolation and analysis of mutations in both plasmid and chromosomal genes involved in the processs. Smaller derivatives of pKM101 will be obtained by restriction-enzyme methodology. The plasmid-coded proteins responsible for the enhancement of mutagenesis will be identified. The biochemical basis of the action of pKM101 will be explored and its relationship to known DNA repair pathways will be determined. The physiology and base-pair specificity of the pkM101-mediated processes will be determined. The Esherichia coli umuC gene will be cloned and analyzed in a similar fashion. An in vitro system will be developed to study the biochemistry of chemical mutagenesis. Results of these studies will be applied to improving bacterial screening systems for carcinogens. It is hoped that elucidation of the molecular mechanisms of chemical mutagenesis in enteric bacteria will provide a model for similar processes in eucaryotes and will lead to an understanding of the origin of diseases such as cancer.

65. CYTOTOXIC, MUTAGENIC AND CARCINOGENIC EFFECTS OF ENERGY RELATED AGENTS IN DIPLOID HUMAN CELLS WHICH DIFFER IN DNA REPAIR CAPACITY

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A quantitative system has been perfected to measure the cytotoxicity of chemicals and radiation in diploid human cells in culture and the frequency of induced mutations. The system has been adapted to diploid cells derived from persons with genetic predisposition to cancer and defective capacity for DNA repair. Studies to date indicate a direct relationship between the cells' capacity to repair damage to DNA and the cytotoxic and mutagenic response and demonstrate that, in contrast to the response of commonly employed aneuploid mammalian cell lines, no measurable increase in mutations is induced by several agents at doses which yield a shoulder on the survival curves of normally repairing cells. Using diploid human fibroblasts with different capacities for DNA repair, we will: (1) investigate the relationship between threshold doses for cytotoxicity and mutagenicity and the ability of cells to repair lesions in DNA caused by low doses of energy-related chemicals and radiation; (2) quantitate the number and kind of chemical adducts remaining bound to the DNA of cells at critical times following exposure to determine the correlation between these adducts and the cytotoxic and mutagenic effects of the chemicals; (3) measure the cytotoxic effect of selected industrial carcinogenic metals in human cells and determine whether they can induce mutations and/or transformation; and finally, (4) investigate the cytotoxic and mutagenic effect of these energy-related agents in diploid fibroblasts derived from a series of selected mammals in order to compare the results with those determined above.

66. RADIATION INDUCED MUTAGENESIS AND CARCINOGENESIS

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The objective of this research is the investigation of the role of DNA repair in radiation-induced mutagenesis and transformation in a mammalian cell line. The effects of stationary holding recovery on the frequencies of these processes is currently under investigation. The characteristics of DNA repair in the cells will be studied by measuring error-free and error prone reactivation of irradiated virus by the host cells under various conditions. We also hope to isolate repair-defective strains following mutagenesis of the cells with ethylmethane sulfonate (EMS) and enrichment using a host-cell viral reactivation suicide technique. Changes in the frequencies of carcinogenesis and mutagenesis in the wild-type cells are compared to the repair-deficient strains will then be correlated to deficiencies in specific repair processes.

67. PHOTO-INDUCED CELL KILLING, MUTATION, AND DNA REPAIR

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This study will measure photochemically induced cell lethality and mutagenesis (6-thioguanine resistance) in normal and xeroderma pigmentosum human diploid cells as a function of wavelength. Selected wavelengths in the far UV, near UV, and visible range will be used.

We will investigate the role of DNA damage and repair phenomena in cell lethality and mutagenesis. At each wavelength, DNA strand breaks, pyrimidine dimers, unscheduled DNA synthesis, and the average site of repair-synthesized DNA regions will be determined. The oxygen dependence of cell lethality, mutagenesis, and DNA damage and repair will also be measured as a function of wavelength. This will allow determination of the contribution of direct and indirect damage mechanisms to these radiation effects.

The overall objectives of this study are to measure lethality and mutagenesis induced by radiations between 254 and 550 nm, and to investigate the basic mechanisms involved.

68. RADIATION AND CHEMICAL INTERACTIONS PRODUCING CELLULAR AND SUBCELLULAR DAMAGES AND THEIR REPAIR

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In order to elucidate mechanisms of chemical sensitization of radiation-induced cellular lethal effects on normal and malignant cells, approaches are being made from the following points of view: 1) Radio-sensitization with iodine compounds such as iodoacetamide or 5-iodouridine might preferentially kill irradiated cells undergoing mutagenesis. This may be useful to eradicate potential cancer cells. 2) Radiosensitization with hypoxic sensitizers of human cells and their repair mechanisms are studied using normal and ataxia cells. 3) A number of typical hypoxic radiosensitizers such as misonidazole are found to be DNA-damaging and mutagenic agents. Their strong toxicities may be due to this chemical background. This problem should be solved by screening other non-mutagenic active chemicals.

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69. SUSCEPTIBILITY OF MOUSE EMBRYOS AND TERATOCARCINOMA CELLS TO AGENTS WHICH DAMAGE DNA

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During the course of the experiments, it was found that the embryos were highly sensitive to irradiation by the incorporated 35S, being killed by exposure to a dose of 0.05 microcurie/ml (MacQueen, Journal of Embryology and Experimental Morphology, 1979, 52, 203). These experiments also revealed that a line of pluripotent teratocarcinoma cells, A6 (which in certain respects resemble embryonic cells), is more radiosensitive than an aneuploid fibroblast line. We are currently extending these studies to examine the sensitiviry of A6 and a line of diploid mouse fibroblasts (WME) to N-methylN'-nitrosoguanidine, an alkylating agent, and to ultraviolet light. Preliminary results indicate that A6 cells are less able to survive damage caused by these agents than WME cells. This could be due to deficiencies in DNA repair processes in the teratocarcinoma cells, which have been shown previously to have a low rate of sister-chromatid exchange (F. Kelly, personal communication). Study of such repair-deficient cells may provide an insight into ways in which normal cells cope with carcinogen-induced damage to their DNA.

70. NATURE AND REPAIR OF A NEW FORM OF DNA DAMAGE

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The role of DNA repair in protecting the cell against mutagens and carcinogens has been suggested by a variety of studies. Most of this work, however, has focused exclusively on the cytobutane pyrimidine dimer form of damage in ultraviolet (uv) irradiated DNA. The overall goal of this project is to extend our understanding of the effects of radiation and chemicals on DNA through the study and use of a damage-specific DNA binding protein isolated from human cells. This protein shows a broad specificity for monofunctional base damage in DNA, but does not recognize pyrimidine dimers, apurinic sites, single-strand breaks, single-strand regions or cross-links in DNA. As well as studying the physical and chemical properties of this protein, we have also utilized this protein as a probe to study the introduction of base damage into DNA by ionizing radiation, alkylating agents and a variety of other chemical mutagens. The role of this protein in cellular repair will be elucidated by studying its interaction with DNA-directed enzymes as well as by screening for its presence in cell lines characterized by abnormal sensitivity to radiation.

71. RADIATION AND CHEMICAL DAMAGE

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The responses of cells to the deleterious agents in the environment that arise from nuclear, fossil and synthetic fuel technologies are governed by the fact that DNA is not only the most vulnerable but also the most important cellular target. Thus cellular responses depend on the radiation and chemically induced changes in DNA and how cells cope with these changes. Our goal is to understand the repair systems in a number of different animals, how they mitigate deleterious effects, and if such systems are inhibited by pollutants from fuel technologies. We will analyze, at the molecular level, the characteristics of the repair steps that take place both before and after DNA replication in bacteria and mammalian cells. We will attempt to correlate biological, enzymological, chemical and physical data on bacterial and various types of mammalian cells so as to identify quantitatively the changes in DNA that have serious biological consequences, and determine how physical and chemical agents affect the repair systems themselves.

72. EXCISION ENZYMES AND THE REPAIR OF DAMAGED DNA

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It is planned to investigate the uvrA, uvrB and uvrC enzymes of E. coli which incise DNA irradiated with ultraviolet light. We aim to purify uvrB protein and to identify and then purify uvrC protein. This will be done with the help of a plasmid carrying uvrB to increase the yield of the uvrB protein, and a lambda transducing phage carrying an amber mutant of uvrC to help in the identification, production and purification of the uvrC protein. Once the uvrC protein has been identified, we will investigate its properties and try to develop methods for its purification.

73. PHOTODYNAMIC DNA DAMAGE AND REPAIR IN HUMAN CELLS

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The two main objectives of the proposed research are: (1) to explore possibilities of improving the present methods of phototherapy of human tumors by studying the basic mechanisms of cell killing using different dye-light combinations and, (2) to accomplish a better understanding of the role of DNA damage and repair in photodynamic carcinogenesis. The present studies will concentrate on an anionic porphyrin, such as hematoporphyrin (Hpd), and, a cationic porphyrin such as the tetraiodide salt of meso tetra (4-N-methyl pyridyl) porphine (4-MPyP), to be used as photodynamic sensitizers in human cells grown in culture. The production of hydroxyl radicals will be measured in human cells that have been exposed to various concentrations of the porphyrins and different levels of light. This will be achieved by determining the yield of (1) thymine glycols in the DNA radioactively labeled in the thymine moiety (methyl-3H).

DNA damage and repair of dye-light treated normal human cells, grown in culture, will be analyzed by measuring single strand breaks and rejoining, using the very sensitive and powerful alkaline elution technique. Such studies will be extended to cancer-prone cells, such as xeroderma pigmentosum, in order to understand the role of DNA repair in photodynamic carcinogenesis.

74. IMPORTANCE OF DNA DAMAGE AND REPAIR FOR CELL SURVIVAL

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The object of this research is to assess the importance of damage to transcribing and nontranscribing DNA sequences for the survival of the mammalian cell. Two different approaches are proposed. In the first we determine whether cells surviving DNA damage have more damage in non-transcribing sequences than transcribing sequences. Cells will be UV-irradiated and the surviving cells harvested at various times. The chromatin is then fractionated and the level of damage assayed in each fraction. An apparently differential loss of damage with time from transcribing DNA could be due to one of two factors - selective survival or preferential repair of this DNA. This will be tested by quantitating the level of repair replication in each fraction. The second approach exploits the fact that there is preferential replication of transcribing sequences in early S phase. The effect on survival of BUdR pulses given at various times throughout S phase will be determined. In addition, a second identical batch of cells will be pulse-labeled with 3H-TdR and the chromatin fractionated. In this way we will have a direct estimate as to the time of replication in S phase of transcribing and non-transcribing sequences, and in addition, we will determine whether BUdR incorporation into transcribing DNA is more damaging to the cell. We have evidence that BUdR incorporation effects the order of replication of DNA sequences and we propose to determine whether the detrimental effect of BUdR incorporation into DNA is mediated through this effect or results directly from DNA damage. The latter can be enhanced by irradiating cells containing BUdR with 313 nm light. For similar levels of survival (with and without irradiation) the effects on the pattern of DNA replication can be compared.

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75. DNA REPAIR IN RELATION TO THE CELL CYCLE

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OBJECTIVE: To explore the mechanism of DNA excision repair and to establish whether it is a regulated phenomenon.

APPROACH: Excision repair of DNA involves the removal of a single-strand patch of DNA containing the lesion. The size of patch varies according to the nature of the damage and presumably, the properties of the repair process involved. All such damage requires DNA repair replication, however, and it is therefore possible to use a wide variety of inhibitors of semi-conservative DNA synthesis to block more or less compietely the synthetic stage of repair. Such agents include hydroxyurea and cytosine arabinoside. The uncoupling of the degradative from the synthetic stages of repair results in the accumulation of enzymically produced single strand DNA breaks. Unwinding the DNA in alkali followed by hydroxyapatite chromatography permits the quantitation of single strand breaks when compared with a sample of cellular DNA X-irradiated and processed in a similar manner. Uncoupling of excision repair enormously amplifies the barely detectable endonuclease-dependent incision events that occur in normal UV repair, and makes it possible to investigate incision mechanisms in intact cells.

PROGRESS: The technique gives a rapid, quantitative and sensitive measure of UV repair activities; less than 1 break per 10 to the ninth power daltons DNA can be measured over a 30 min. uncoupling period. In addition we find that the incision step is preceded by an event reversibly inhibited by novobiocin, an inhibitor of bacterial gyrase and eukaryote DNA replication.

76. EFFECTS OF RADIATION ON STATIONARY CELLS

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The long-term goal of this research program is to gain knowledge about mechanisms which determine the radiosensitivity of mammalian cells, particularly those involved in the response of stationary or very slowly proliferating cells to X-irradiation. We will utilize three different types of cells: 10T1/2 mouse embryo fibroblasts, human diploid cells and human tumor cells. Four cellular endpoints will be studied in parallel: survival, mutagenesis, and the induction of chromosome aberrations and sister chromatic exchanges (SCE). We will investigate the relationships between these cellular effects, and try to correlate them with molecular DNA repair processes. Techniques for measuring DNA repair will include host cell reactivation of irradiated herpes virus, alkaline elution assay for DNA strand breaks and crosslinking, and measurement of endonuclease sensitive sites.

Cellular and molecular phenomena will be studied during the repair of potentially lethal damage (PLD) in stationary phase 10T1/2 and human diploid cells treated with X-rays, UV light or mitomycin-C. The frequencies of induced mutations, SCE and chromosome aberrations will be studied in parallel experiments, and correlated with molecular repair. In particular, evidence will be sought which implicates the action of error-prone repair processes in the cellular effects induced by these agents. Further information on mechanisms will be obtained through the study of skin fibroblasts isolated from individuals who are unusually susceptible to the development of cancer, especially radiation induced cancer, either because of family history or because they have a recognized genetic syndrome associated with an increased frequency of spontaneous and/or induced cancer. We will measure the sensitivity of these cells to the cytotoxic and mutagenic effects of radiation in vitro (mutations to 6-Thioguanine resistance) during PLD recovery, and seek evidence for molecular DNA repair defects. Finally, rapid split dose recovery and PLD repair will be studied in plateau phase human diploid and human tumor cell lines, and a relationship between processes and tumor radiocurability in vivo sought.

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77. RADIATION AND CHEMICAL IN VITRO MALIGNANT TRANSFORMATION

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The basic objective of this research program is to gain specific information about the mechanisms of radiation carcinogenesis using in vitro assay system. Previous results indicate that X-ray transformation in vitro can be thought of as involving at least two steps: 1) the fixation of the transformed state (presumably irreversible DNA damage) and 2) its subsequent expression in terms of morphologically altered cells. We will investigate in detail the conditions necessary for fixation and ultimate expression of the cellular damage induced by ultraviolet light and ionizing radiation which results in malignant transformation of cells. In particular, we plan to study the role that DNA repair processes play in radiation-induced transformation and the interactions between radiation and chemical agents in malignant transformation. At present, little information is available on the interactions between radiation and other agents from in vivo carcinogenesis studies or in vitro transformation work, although human populations are continually being exposed to a variety of carcinogenic agents as well as to other agents that can influence carcinogenesis.

The cell system we will use is an established mouse embryo cell line which has been used for several years in this laboratory for radiation transformation experiments and is particularly appropriate for studying interactions between radiation and chemical agents. The specific chemical agents we wish to study have known interactive effects on carcinogenesis in vivo; studies in an in vitro system in which cell proliferation and other environmental conditions can be precisely controlled should yield further information about the mechanisms of carcinogenesis.

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78. DNA REPAIR AND RECOVERY IN THE MAMMALIAN CELL CYCLE

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The ultimate objective of this research is to determine the biochemical basis of cell cycle-dependent variations in the cytotoxicity and mutagenicity of radiation and chemical agents, and to define the relationship of certain DNA repair processes to mammalian cell survival and mutagenesis. We propose to determine: a) the extent to which cell cycle-dependent variations in cell killing by mutagenic agents are paralleled by variations in induced mutation frequencies, b) whether cell killing and mutation induction by such agents display qualitatively similar dose-response kinetics, and c) the effects of specific DNA repair processes, dose-fractionation and inter-dose recovery time on the kinetics of repair and ultimate expression of potentially-lethal versus potentially-mutagenic lesions. A second major objective of this research is to isolate and characterize a broad spectrum of different mutagen-sensitive variants of CHO cells that could be used to investigate the molecular and genetic bases of DNA repair and somatic cell mutation.

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79. EFFECTS OF RADIATION ON HUMAN CELLS CULTURED IN VITRO

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The proposed research is a study of the effects of low doses (sublethal) and repeated exposure to low doses of ultraviolet (UV, 254 nm) and ionizing radiation (X-rays) on cultured human diploid fibroblasts (HDF). Current emphasis is on defining the effects of these radiations on arrested nonmitotic populations of various HDF strains including the DNA repair-deficient xeroderma pigmentosum (XP) and ataxia telangiectasia (AT) strains and determining the rate and extent of DNA repair in these arrested populations. Another emphasis is on inducing with these radiations HDF transformants in culture.

A fraction of cells from UV-irradiated arrested populations is lost upon subsequent incubation. The extent of cell loss is dose dependent and cell strain specific. Xeroderma pigmentosum strains are more sensitive to UV than are normal HDF. The sensitive target is DNA. The lethal event appears to be one on RNA transcription that leads to an inhibition of required protein synthesis. DNA excision repair proficient strains are more resistant to UV because they can remove to some extent the DNA damage that leads to an effect on RNA synthesis. Experiments to study the rate and extent of DNA repair in both UV and X-irradiated normal, XP, and AT populations of nondividing cells are proposed. The assay for repair is the loss of DNA sites sensitive to UV-specific or gamma-specific endonuclease. The objective is to gain from these rate studies an understanding of the mechanisms of excision repair in normal cells and some insight into the repair defects in the other strains.

Other experiments to induce and detect HDF transformants in culture are proposed. Emphasis is placed on using HDF strains isolated from individuals with a predisposition to cancer, using fresh primary isolates, using single and multiple exposures to X-rays and UV with chemicals defined as cocarcinogens (phorbol esters, diethylstilbestrol, anthralin) and culture procedures that permit expression of a transformation event.

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80. THE ROLE OF DNA REPAIR IN THE THERAPEUTIC RESPONSES OF MELANOMA

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Melanomas are generally considered to be resistant to radiation therapy. They also respond poorly to chemotherapy. The reasons for their poor response have not been clearly defined.

OBJECTIVE: To determine the role of DNA repair in the response of melanomas to chemo- and radiation therapy, in the development of resistance to therapy and in metastasis.

APPROACH: The studies will be performed using mouse cells. Rodent cells, in general, perform excision repair at low rates, thus, the specific role of post-replication repair (PRR) will be studied in the absence of interference from concomitant excision repair. Responses of 3 transplantable melanomas (B16, Harding-Passey and Cloudman S91) will be compared to those of a transplantable mammary carcinoma (8EMT6) and to a normal cell line (NCL). The 4 tumor lines grow well as solid tumors and at high plating-efficiency in vitro.

PREVIOUS FINDINGS: PRR rates in the malanomas are about twice that of the normal mouse lung fibroblast line (MLF) studied earlier. The PRR rate of EMT6 is about 50% higher than that of MLF. Excision repair rates do not differ significantly in the melanomas as compared with MLF.

CURRENT PLANS: Natural and inducible PRR activity in the cell lines will be correlated with UV, x-ray and chemotherapeutic agent sensitivity to killing, with repair of potentially lethal damage, and with spontaneous and induced mutation rates. PRR activity of highly metastatic lines will be compared with that of weakly metastatic ones. PRR activity of drug-resistant mutants will be compared with that of weakly metastatic ones. PRR activity of drug-resistant mutants will be compared with that of of the drug sensitive wild-type. The role of gene dose in increasing the rate of PRR in the melanomas will also be examined.

81. DNA REPAIR DEFICIENCY AND INCREASED PROBABILITY OF CANCER IN CERTAIN HUMAN GENETIC DISEASES

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Studies comparing the post-treatment behavior of fibroblasts from patients with ataxia telangiectasia with that of fibroblasts from control donors showed then similar behavior after either methyl methane sulfonate (MMS) or ultraviolet (UV) treatment, but different behavior (decreased colony forming ability and usually decreased DNA-repair synthesis) after N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatment.

Similar studies using a number of human tumor cell strains showed many of these to be extremely sensitive to MNNG-produced killing, somewhat sensitive to MMS and BCNU (bis-chloroethyl-nitrosourea) killing, but not to UV-produced killing.

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82. DNA REPAIR IN HUMAN CANCER-PRONE GENETIC DISEASES

Kraemer, K. H., Scudiero, D. A., Levis, W. L., Greene, M. H., Kohn, P. H., Stinson, S. F., Cohen, L. F., Gelboin, H. V., Bader, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Genetics Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Human cancer-prone genetic diseases are being studied in order to identify groups of people with an increased susceptibility to environmental carcinogenesis, and to attempt to correlate such sensitivity with clinical features and with defective DNA repair. Patients with xeroderma pigmentosum and ataxia telangiectasia, diseases with ultraviolet and X-ray sensitivity, respectively, and with familial malignant melanoma (dysplastic nevus syndrome), and neurofibromatosis, diseases with no documented environmental sensitivity, are being studied. Detailed examinations of the clinical features of affected individuals are being made. Cultures of skin and blood are being established and the effects on cell survival, DNA repair, and chromsome abnormalities after treatment with DNA damaging agents (ultraviolet, bleomycin, and psoralen plus long wavelength ultraviolet) are being examined.

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83. DNA DAMAGE AND REPAIR IN IRRADIATED BRAIN AND BRAIN TUMORS

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The objective of this research is to elucidate the molecular mechanisms responsible for the differences in DNA repair kinetics observed in normal cells (brain, lymphocytes, macrophages) and tumor cells after irradiation or treatment with drugs. Our previous work indicates that 1) normal nondividing neurons repair radiation-induced DNA damage at a much slower rate than dividing brain tumor cells, 2) structural changes in the DNA of neurons can be used to evaluate the long-term effects of brain irradiation, and 3) recovery from potentially lethal damage is a major determinant of radiocurability in 9L brain tumors. We propose to use either the alkaline elution technique

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of Kohn or our alkaline sucrose gradient technique in slow reorienting zonal rotors to define the DNA damage and repair kinetics after treatment with radiation, drugs, or combinations of drugs with radiation in tumors and normal cells isolated from or near the tumors. The tumor model will be either intracerebral or subcutaneous 9L tumors in Fisher 344 rats. Normal cells will be 1) cerebellar neurons, or 2) lymphocytes and macrophages isolated by centrifugal elutriation from 9L tumors. The alkaline elution analysis will determine the relative amount of DNA-DNA crosslinks, DNA-protein crosslinks and DNA strand breaks in treated cells. Repair kinetics will be evaluated by the relative rates and extent of removal of these different types of damage. Differences in the DNA repair kinetics among the various cells studied may be due to differences in the DNA template structure or differences in the availability or function of the repair enzymes. The susceptibility of chromatin isolated at various steps of the repair process to digestion by nucleases or its ability to support polymerase activity will be used to evaluate the template theory. Assays for alpha and beta polymerase activity from both normal and malignant cells will be used to test the repair enzyme theory. We will attempt to determine whether critical periods in the DNA repair process are associated with changes in either the DNA template structure or the quantity or quality of the repair enzymes.

84. VISCOELASTIC ANALYSIS OF BRAIN AND TUMOR DNA

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This research project is concerned with the viscoelastic behavior of mammalian cell DNA. We are examining the conformational properties of mammalian DNA from cells that have been treated with gamma rays and various drugs in an effort to characterize the DNA damage resulting from such treatment. Repair of the various lesions introduced by gamma rays and drugs is also being investigated. The types of damage under study include single-strand and double-strand breaks and DNA-DNA crosslinks.

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85. STUDIES OF DNA REPAIR IN HUMAN DEGENERATIVE DISEASES

Robbins, J. H., Moshell, A. N., Scarpinato, R. G., Snowden, K. I., Potkay, S., Tarone, R. E., Doniger, J., Scudiero, D. A., Lytle, C. D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Dermatology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Studies in this laboratory are designed to elucidate the role of DNA repair processes in huamn diseases and in carcinogenesis, mutagenesis, and normal and abnormal aging. Most studies have been conducted with cells from patients with xeroderma pigmentosum (XP), all of whom have defective DNA repair plus the clinical abnormalities of multiple cutaneous malignancies and premature aging of sun-exposed skin. Studies are currently being performed on cells from patients with degenerative neurological diseases such as Huntington's disease, ataxia telangiectasia, familial dysautonomia, olivopontocerebellar atrophy, and Cockayne's syndrome. These studies are designed to elucidate the pathogenesis of these disorders as well as to develop presymptomatic (including prenatal) diagnostic tests. We assess the biological effectiveness of DNA repair primarily by in vitro assays of post-irradiation colony-forming ability and trypan blue exclusion. We are extending our work to include cells from patients with other degenerative disorders including Shy-Drager syndrome, idiopathic orthostatic hypotension, retinitis pigmentosa, Usher's syndrome, spinal muscular atrophy, and muscular dystrophy.

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86. GENETIC STUDIES OF CELLULAR RESISTANCE TO PHYSICAL AND CHEMICAL CYTOTOXIC AGENTS

Boyle, J. M., Durrant, L. G., Paterson Lab., Dept. of Experimental Chemotherapy, *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVE: To investigate dominance and complementation of genetic factors affecting cell survival and mutagenicity in cell lines with differential sensitivity to cytotoxic agents.

APPROACH: 1. Pairs of rodent tumor cell lines have been chosen which appear to show differential sensitivity to a specific DNA damaging physical (UV or X-rays) or alkylating (e.g., MDMS) agent. 2. The patterns of cross-sensitivities to other cytotoxic agents are being determined. 3. Mutation induction is being measured for thioguanine and ouabain resistances, and will provide marked cell lines for fusion studies. 4. Cell survival, mutagenicity and karyotypes of hybrid cells will be compared with those of parent cells. 5. Where complementation patterns are found to give clearly distinguishable classes, biochemical characterization of DNA repair may be possible. 6. Where practicable, human and rodent fusions will be made which may provide material for future mapping studies.

87. SEROLOGIC ASSAY OF DNA BASE AND REPAIR FOLLOWING HYPERTHERMIA AND RADIATION

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The aim of this research is the elucidation of the mechanism of synergism between hyperthermia and radiation. We have shown that acute hyperthermia (45 degrees C, 15 min.) completely blocked removal of UV photoproducts (serologic determination). UV radiation survival of cells was not affected, however, whether heat was administered post- or pre-irradiation. These cells (HeLa-S, Dr. G. Sato, UCSD) did exhibit synergism between ionizing radiation and post-but not pre irradiation hyperthermia. Since UV results in base damage while ionizing radiation produces both base damage and strand breaks, the observed difference in response of UV and ionizing radiation treated cells to hyperthermia could be due to strand breaks. Synergism might result from heat inactivated repair of strand breaks but not base damage. To test this hypothesis, cells were labeled with BUdR prior to treatment. This should result in UV induced strand breaks and a response similar to that found in ionizing radiation. Although the expected sensitization to UV was observed, no synergism occurred when cells were also heat treated post-UV radiation. There was no BUdR sensitization to heat alone. Similar results have been obtained with CHO cells. Direct measurement of strand break production/repair by alkaline elution is underway. Lymphoma cell lines (S49 and variants, Dr. P. Coffino, UCSF) are being established in lab to test other possible mechanisms involving heat effects on control of metabolism. Serologic determinations have been delayed due to power failure and loss of materials but will resume with emphasis on ionizing damage and photoproducts. Some of this work is in preparation for publication.

88. DNA SYNTHESIS AND REPAIR STUDIES ON INITIATION SITES AND GROWTH OF REPLICONS IN RELATION TO CHROMATIN STRUCTURES

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The summary for this project is under entry no. 6.

89. DNA STRUCTURES WHICH STIMULATE GENETIC RECOMBINATION AND DNA REPAIR

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The summary for this project is under entry no. 7.

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90. REPAIR OF RADIATION-INDUCED LESIONS IN DNA

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OBJECTIVES: (1) We plan to confirm our preliminary work with E. coli K-12 that, with UV radiation, back mutations occur by a one-hit process, and suppressor mutations occur by a two-hit process. (2) As controls for previous mutation studies, we observed that the different DNA repair deficient strains showed different levels of spontaneous mutagenesis in the same relative manner that these repair genes controlled UV radiation mutagenesis. To examine this phenomenon in more detail, we have studied a reversion system, but now need to study some forward mutations. We will also test various growth conditions for their possible effects on the level of spontaneous mutagenesis. (3) The recB, lexA and uvrD genes appear to act additively for survival after UV irradiation, while the recF mutation acts synergistically with these mutations. We have a new mutation (radA) that sensitizes a uvrB strain. We will test if this gene functions in the recF or the recB, uvrD, lexA pathways of postreplication repair. We will also study the effect of the radA and recF genes on the UV radiation-induction of resistance to UV radiation.

91. EFFECTS OF RADIATION ON THE VIRUS/CELL INTERACTION

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OBJECTIVE: To examine the effects of radiation exposure on the interaction between adenovirus and mammalian cells. The objective is to determine the role of radiation-induced DNA damage, DNA repair mechanisms, and viruses in human carcinogenesis.

APPROACH: Host-cell reactivation (HCR), enhanced reactivation (ER) and enhanced mutagenesis (EM) of adenovirus are being examined in a variety of mammalian cells, including normal human cells, cells from patients with a predisposition to cancer, virally transformed cells, spontaneously transformed cells and cells with suspected repair defects. HCR and ER of irradiated adenovirus in human fibroblasts are being studied by examining the production of viral structural antigen using immunofluorescent staining. EM is being studied by examining the reversion frequency of adenovirus temperature sensitive mutants following the infection of human fibroblasts with the irradiated virus mutants. The effects of radiation on viral transcription and the synthesis of viral proteins are also being examined.

PROGRESS: A reduced HCR for irradiated virus (either UV or gamma or both in some cases) has been found following infection of some fibroblasts from patients with xeroderma pigmentosum (XP) (both excision deficient variants and heterozygotes), Cockayne's syndrome (CS), Fanconi's anaemia, progeria, Huntington's chorea, Bloom's syndrome and ataxia telangiectasia. UV or gamma irradiation of normal human fibroblasts prior to infection leads to an enhanced reactivation of both UV and gamma irradiated adenovirus. Radiation enhanced reactivation or irradiated virus also occurs in some XP and CS cell strains. Reports on some of this work have been published (Biochem. Biophys. Res. Commun. 90, 567, (1979); Mutation Res. 60, 31, (1979) and 69, 357, (1980); Ant. J. Radiat. Biol. 36, 621, (1979). Cancer Research 40, 3945, (1980). Radiat. Res. 83, 475, Ge-7 and Ge-8, (1980).

92. DNA REPAIR IN SLIME MOLD

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We will study the enzymatic repair of damage to the DNA of replicating and developing cells of the cellular slime mold, Dictyostelium discoideum, after treatment with certain mutagenic and carcinogenic agents, including ionizing radiation, ultraviolet light, and alkylating chemicals. Repair at various stages of development will be compared to that in the replicating vegetative cells. The genetics and biochemistry of repair in a series of repair-defective mutants will be characterized using parasexual genetic techniques, specific-enzyme monitoring of DNA damage, in vivo appearance and resealing of breaks in DNA, repair replication via density-shift techniques, BUdR photolysis of repair patches, and in vitro analysis of specific repair-enzyme activities extracted from these cells. Mutagenesis assays will quantitate mutation production in these strains with above agents administered during growth and development. Correlations will be made between mutagenesis and repair of specific lesions. Some emphasis will be placed on mutagenesis by: (1) ionizing radiation, highly mutagenic in this organism and capable of readily penetrating the developmental aggregates, and (2) nitrosoguanidine, an alkylating agent that produces relatively large amounts of 6O-methyl-guanine and shows preferential mutagenesis in replicating regions of DNA. Effects of repair modifiers such as caffeine and treatment fractionation on mutagenesis will be studied. In order to improve the introduction of isotopic labels, nutrients, inhibitors, and other molecules into these amoebae, we will investigate feeding and injection regimes utilizing loaded membrane vesicles.

93. DNA REPLICATION AND THE REPAIR OF DNA STRAND BREAKS IN NUCLEI OF PHYSARUM POLYCEPHALUM

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This research combines biochemical and genetic experimentation in an effort to increase our knowledge of DNA replication and repair. The objectives are to develop an in vivo DNA replication system that faithfully carries out the same replicative processes that have been escribed in vivo; to study both replication and repair of DNA using the in vitro system; to obtain conditionally-mutant strains of P. polycephalum that are specifically defective in DNA metabolism; and to use these strains in in vitro systems in order to define gene products necessary for DNA replication and repair. The approach includes: in vivo and in vitro measurements of DNA replication intermediates using alkaline sucrose gradient centrifugation; measurement of rate and amount of incorporation of radioactively-labeled precursors into newly-synthesized DNA; isolation of temperature-sensitive amoebae; construction of plasmodia from the ts amoebae; and characterization of the plasmodia as to possible defects in DNA metabolism. The expected result is a description at the molecular level of DNA replication and repair in this eukaryote. Two DNA polymerases from Physarum polycephalum have been separated by sucrose density gradient centrifugation. A heat-stable glycoprotein prepared from nuclei stimulates in vitro DNA synthesis in nuclei. Radiation-induced DNA double-strand breaks are rejoined in intact plasmodia and isolated nuclei in a cell cycle-dependent manner. Strand breaks induced by the carcinogen 4-nitroquinoline-1-oxide are similarly repaired. Temperature sensitive plasmodia of P. polycephalum have been constructed and preliminary genetical and biochemical characterizations have been carried out. Two of the strains appear to be conditionally defective in DNA metabolism. An isogenic ploidal series of amoebae has been prepared and characterized as to uv and ionizing radiation sensitivity.

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94. RADIATION REPAIR IN DROSOPHILA MELANOGASTER

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Drosophila melanogaster, a genetically well-characterized organism, is especially suitable for the elucidation of the biochemical basis of DNA repair mechanisms in eukaryotes. Since the various modes of DNA repair are intimately related to DNA replication, mutagenesis, and recombination; the enzymes involved in the correction of DNA damages may be instrumental in the carcinogenic process. The proposed research is designed to screen for specific repair endonuclease activities in wild-type Drosophila and in various known repair-deficient mutants. In order to assay for such damage-specific endonucleases,

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DNA substrates will be prepared by treatment with ultraviolet light, X-rays and alkylating agents.

95. DNA REPAIR STUDIES IN XERODERMA PIGMENTOSUM CELLS

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Xeroderma pigmentosum (XP) is a hereditary disease in humans. It is characterized by extreme sensitivity to sunlight and to sun-induced skin disorders such as actinic damage and cancer. Cultured cells from XP patients have defective DNA repair mechanisms and are more sensitive to UV-light than are cells obtained from healthy individuals. The specific DNA repair defect in XP cells has not been characterized, but ample evidence suggests that it may be in the incision step of the nucleotide excision-repair pathway. The objectives of this research are to: (1) examine the abilities of sonicated, cell-free extracts from different XP complementation groups and from XP variant cells to excise thymine dimers from their endogenous chromatin, (2) perform in vitro complementation experiments with XP complementation groups that are shown to be defective in excision repair, and (3) investigate the dimer-excising ability of normal and XP cell-free extracts prepared by methods other than sonication.

96. DNA REPAIR PROCESSES IN XERODERMA PIGMENTOSUM

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Mutations in at least eight genes affecting the repair of ultraviolet-induced DNA damage have been identified among patients with xeroderma pigmentosum. The proposed studies will compare the responses of cells from normals and from patients with these different forms of xeroderma pigmentosum to ultraviolet damage. The studies have five interrelated objectives: 1) To determine the relationship between DNA repair capacity and the cell cycles, by studying colony-forming ability following ultraviolet radiation in synchronized fibroblast cultures derived from patients with xeroderma pigmentosum. 2) To identify the portion of the UV spectrum (i.e., the action spectrum) responsible for the photochemical damage to which patients with xeroderma pigmentosum are susceptible. This will be accomplished by studying cell survival following irradiation with different ultraviolet sources. 3) To assess the possible interactions of different wavelengths of UV in the repair processes of cells from normal and xeroderma pigmentosum patients, by comparing the effects of combined and separate irradiations with different portions of the UV spectrum. 4) To assess the role of 'inducible' (and presumably mutagenic) repair processes in cells of normal and xeroderma pigmentosum patients, by seeking to demonstrate an enhanced survival of ultraviolet-irradiated cells following the administrations of small 'inducing' doses. 5) To continue the identification and characterization of new repair mutations in xeroderma pigmentosum patients, by determining repair synthesis rates, complementation groups, and ultraviolet sensitivity.

The overall goal of these studies is to increase our understanding of the relationship between human DNA repair and environmentally-induced diseases of the skin and other organs.

97. THE RELATIONSHIP BETWEEN CARCINOGENESIS AND DNA REPAIR IN XERODERMA PIGMENTOSUM HETEROZYGOTES

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Ultraviolet light-induced DNA repair rates will be measured in a number of fibroblast lines that are obligate heterozygotes for xeroderma pigmentosum (XP). XP is a skin-cancer-prone, autosomally recessive human disease. Homozygotes for XP are defective in repairing UV-induced DNA damage.

Our preliminary results, obtained using an M. luteus endonuclease assay for DNA repair, indicate reduced repair, relative to normals, in all 8 obligate XP heterozygote lines that we have tested. In addition, a reduced repair rate was found in one unaffected sibling of an XP family. These heterozygotes include representatives of complementation groups A through D, as well as several unclassified lines.

We will extend our study to 15-20 additional heterozygote lines. We will measure: excision resynthesis with DND-cellulose chromatography, removal of endonuclease-sensitive sites, repair of N-acetoxy-2-acetyl-aminofluorene (AAAF) induced DNA damage and, in obligate heterozygote parents of XP 'variants', we will measure levels of post-replication repair.

Our goals are to: 1) further document the existence of DNA repair deficiencies in XP heterozygotes. 2) Compare repair rates in heterozygotes with skin cancer frequencies in blood relatives. This skin cancer data is now available from studies by Dr. M. Swift of skin cancer frequencies in XP families. 3) Investigate aspects of the genetic complexity of XP through analysis of intrafamilial variations in repair. Our results may promote a clearer understanding of the role of DNA repair genes in human carcinogenesis.

98. POST-REPLICATION-REPAIR OF DNA IN NEUROSPORA

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The fungus Neurospora crassa is a very useful model for DNA-repair studies in eukaryotes because it is easy to maintain, to study genetically, and to handle biochemically. Using zymolyase lysis of cells on top of alkaline sucrose gradients, we have shown that the pyrimidine dimer excision-defective uvs-2 strain has a post-replication-repair system very similar to that of other eukaryotes. After ultraviolet-light irradiation, DNA synthesis is depressed and DNA is made in short segments for an abnormal length of time, due to blockage of synthesis by dimers. Eventually, the blockage is overcome and normal length DNA is made, although dimers remain in the template and DNA synthesis remains depressed. Caffeine slows, but does not block recovery. The process does not involve recombinational transfers of dimers to newly made DNA. Unlike other eukaryotes there may be gaps at the site of pyrimidine dimers before they are bypassed. We plan to examine mutagen sensitive mutants for blocks in this system and to examine replicon length and initiation patterns in Neurospora chromatin.

These results validate the use of Neurospora for mutagenesis studies and should help uncover the mechanisms underlying this repair system which is thought to be fundamental in mutation and cancer induction.

99. INDUCTION AND REPAIR OF DNA DAMAGE AFTER IRRADIATION OR TREATMENT WITH ANTI-CANCER DRUGS

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DNA is a critical target molecule for many agents such as ionizing radiation, many mutagenic and carcinogenic substances as well as for many anti-cancer drugs. We have started a clinical study of the induction and repair of DNA strand breaks in human lymphomas after irradiation or after treatment including anti-cancer drugs. Our aim is to find possible correlations between the primary damage - DNA strand breaks or unrepaired breaks - and the final results of an irradiation treatment or anti-cancer drug treatment. We hope that by studying the primary damage we can arrive at an early prognosis of the results of the treatment.

Cell samples are taken from the tumor by fine needle aspiration. They are used for cytological examination and for the analysis of DNA strand breaks. Cells are embedded into agarose gel and after alkali treatment stained using acridine orange (AO). The fluorescence at 525 nm (green, from AO bound to double-stranded DNA) and abone 610 nm (red, from AO bound to single-stranded DNA) is measured in a microscope photometer. The ratio of the values obtained at 610 nm to those obtained at 525 nm is a measure of the number of DNA strand breaks (one of the critical damages) in the cell.

100. REPAIR OF RADIATION DAMAGE IN VITRO AND IN VIVO

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tal & Tumor Inst., Dept. of Physics, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

The objective of this research is to understand in detail the mechanisms by which mammalian cells repair ionizing radiation damage in their DNA. Relationships between the repair of lesions in DNA at the molecular level and cellular radiobiological phenomena which are believed to be important to human cancer radiotherapy (including the variation in sensitivity through the cell cycle, the oxygen effect, the increased effectiveness of high linear energy transfer (LET) radiation, the differences in radiosensitivity between tumor cells and normal cells) will be investigated. The experimental approaches to these questions will make use of several techniques which have become available in the last few years, such as synchrony of mammalian cells by centrifugal elutriation and detection of strand breaks in DNA by alkaline elution.

While these methodologies were originally developed for use on cultured mammalian cells, they have recently been modified so that cells from normal or tumor tissue may also be studied. Thus, the questions concerning the importance of DNA repair at the molecular level to radiobiological phenomena at the cellular level may now be addressed in the in vivo tumor situation. Ultimately, knowledge about these relationships may allow the design of radiotherapy modalities that take maximum advantage of radiobiological factors.

101. RADIATION DOSE RATE EFFECT ON REPAIR DEFICIENT CELLS

Dritschilo, A., Georgetown University, School of Medicine, Dept. of Radiology, *3800 Reservoir Rd. N.W., Washington, District of Columbia,* 20007, United States of America

We will study the effects of ionizing radiation at low dose rate on human diploid fibroblasts from patients with known or suspected DNA repair defects. The responses of these cells (ataxia-telangiectasia, xeroderma pigmentosum and Fanconi's anemia) will be compared to the response of normal human fibroblasts. Radiation damage-time interactions will be studied as these are expressed by survival, i.e., capacity to divide without limit and repair capacity. Two culture conditions will be used: 1) exponential and 2) plateau phase of growth.

C. DNA REPAIR AFTER DAMAGE BY CHEMICAL CARCINOGENS

102. DNA DAMAGE AND REPAIR - TRANSPLACENTAL CARCINOGENESIS - MORPHOLOGICAL PARAMETERS OF TRANSFORMED CELLS (ABBREV)

Santi, L., Cesarone, C. F., Bolognesi, C., Nicolo, G., Grondona, G. F., Margano, E., Rossi, L., Barbieri, O., Valerio, F., Istituto Scientifico per lo Studio e la Cura dei Tumori, *Viale Benedetto XV,10, 16132 Genoa, Italy*

In vivo DNA damage and repair evaluation: Screening of different classes of chemical compounds with reference to physical and chemical DNA damages obtained using standard agents. Using X-rays we have standardized the DNA damage at various dose levels in order to use these damages as reference for chemical treatments. The behavior of aromatic amines and their metabolites is being investigated. DNA repair can be also evaluated along with the damage induced by the treatment of animals with this class of compounds. The alkaline elution technique has been innovatively and greatly improved by the application of a new method for the fluorometric evaluation of DNA at nanogram level.

Transplacental carcinogenesis: The overall objective of our experiment is to investigate the possible carcinogenic activity of Phenobarbital-Na (Ph-Na) and Phenytoin-Na (Pht-Na) in Swiss mice, via transplacental exposure. A pilot test has shown that Ph-Na at a dose of 0.64 mM/Kg and Pht-Na at a dose of 0.32 mM/Kg are embryotoxic and teratogenic (cleft palate and limb deformities). For the main experiment, groups of 10 females each were treated daily from day 1 to term of pregnancy with Ph-Na 0.25 mM/Kg and Pht-Na 0.10 mM/Kg via s.c. injections. Further groups of 10 females each were treated daily from day 12 to term of pregnancy with Ph-Na 0.40 mM/Kg and Pht-Na 0.20 mM/Kg via s.c. injections. Controls were treated with the solvent of Pht-Na (50% saline, 40% propylene glycol and 10% ethanol) or saline. Newborn are now 5-6 weeks old. Other experimental groups have been prepared to study the combined effects of Ph-Na and Pht-Na in transplacental carcinogenesis, the role of male factors (males will be treated with the drugs before mating) and the effects of the drugs directly injected into the amniotic space of the mouse.

Analytical and quantitative cytology of in vitro transformed, and human tumor cells: Diagnostic and prognostic classification and therapeutic value of ploidy determinations in tumor cells as an adjuvant in oncology and in carcinogenesis study. DNA static cytophotometry at high resolution by epifluorescence and scanning procedure on line with an automatic calculator HP 21 MX. Study of the biologic behavior of nuclear chromatin and its degree of condensation or dispersal in the tumor cell. (Text Abridged.)

103. THE INVOLVEMENT OF POLY(ADP-RIBOSE) AND DNA DAMAGE IN ERYTHROLEUKEMIC DIFFERENTIATION

Skidmore, C. J., University of Reading, Dept. of Physiology & Biochemistry, *Whiteknights, Reading RG6 2AB, England, United Kingdom*

OBJECTIVE: To define temporarily and chemically the alkali-sensitive lesions in cellular DNA which arise during Friend cell differentiation and to investigate their importance.

APPROACH: The alkali-labile lesions which arise in the DNA of DMSO-induced Friend leukemia cells will be defined with respect to markers of nuclear activity - DNA and RNA synthesis and cell-cycle parameters. Particular attention will be paid to the relationship between DNA lesions and poly(ADP-ribose) polymerase - an enzyme sensitive to DNA damage. The interdependence of the markers will be investigated by the use of selected inhibitors of these processes.

The nature and persistence of the DNA lesions will be examined by methods developed for DNA repair studies including sucrose gradient analysis and use of specific endonucleases.

104. SUSCEPTIPILITY OF MOUSE EMBRYOS AND TERATOCARCINOMA CELLS TO AGENTS WHICH DAMAGE DNA

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The summary for this project is under entry no. 69.

105. DNA DAMAGE - HUMAN LYMPHOID CELLS

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Two different pathways for excision repair occur in mammalian cells: (1) nucleotide excision, and (2) base excision (apurinic) repair. The activity of both is controlled by cellular regulatory mechanisms. We propose: (1) to determine the pathway for the excision of polyaromatic hydrocarbon adducts from DNA using human lymphoblastoid cells; (2) to establish rodent liver cell cultures and determine their repair activity for the two pathways; (3) to compare the repair activity of rodent liver cells with other rodent cells including neuroblastomas and gliomas in order to determine whether the sensitivity of neutral tissue to carcinogenesis is related to a repair deficiency in a specific pathway; (4) to characterize a new reaction in which DNA with polycyclic aromatic hydrocarbon residues binds to columns of BND-cellulose; and (5) to continue our studies on the mechanism by which DNA synthesis is able to bypass lesions.

BIBLIOGRAPHIC REFERENCES: Tatsumi, K., and Strauss, B., Production of DNA Bifilarly Substituted with Bromodeoxyuridine in the First Round of Synthesis: Branch Migration During Isolation of Cellular DNA, Nucleic Acids Research 78: 331-347 (1978); Bose, K., P. Karran and B. Strauss, Repair of Depurinated DNA In Vitro by Enzymes Purified from Human Lymphoblasts, Proc. Natl. Acad. Sci. U.S. 75: 794-798 (1978).

106. NATURE AND REPAIR OF A NEW FORM OF DNA DAMAGE

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The summary for this project is under entry no. 70.

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107. DNA DAMAGE BY CHEMICAL CARCINOGENS AND ITS REPAIR IN HUMAN CELLS

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Our initial studies have involved the effect of asbestos and several metal carcinogens, which are often present in trace levels in asbestos, on DNA in human cells. With the alkaline elution technique, DNA-protein crosslinks were detected in fibroblasts exposed to chromate salts. No evidence of repair was seen with up to 12 hours repair incubation after chromate removal from the culture medium. Using an in vitro assay, DNA-protein crosslinks were also detected. In the case of asbestos and nickel, no DNA damage was seen after exposure of fibroblasts to these agents. With the development of methods to maintain human bronchial epithelial cells in culture, DNA damage and repair studies are now being initiated with these cells with particular emphasis on agents implicated in pulmonary carcinogenesis.

108. ROLE OF PHARMACOKINETIC, METABOLIC AND CHEMICAL PARAMETERS IN THE INDUCTION OF DNA DAMAGE

Daniel, F. B., U.S. Environmental Protection Agency, Office of Research & Development, Health Effects Research Lab., *5555 Ridge Ave., Cincinnati, Ohio*, 45268, United States of America

OBJECTIVE: To elucidate the role of pharmacokinetic and metabolic parameters in the rate and extent of DNA damage induction by chemical carcinogens in vivo.

APPROACH: Rodent strains sensitive (rat) and resistant (mouse) to the hepatocarcinogen aflatoxin B1 will be evaluated for rate of metabolism and induction of DNA damage in target and non-target tissue.

109. DNA DAMAGE IN HUMAN LYMPHOCYTES AS INDICATORS OF CARCINOGEN EXPOSURE

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OBJECTIVE: To measure the severity and persistence of DNA damage in human peripheral lymphocytes after administration of antineoplastic agents.

APPROACH: Adriamycin and cyclophosphamide will be administered to mice at the same dose those given to newly admitted cancer patients. Cancer patients previously treated with the drugs will be evaluated to determine the persistence of DNA damage in human tissue.

110. ASSAY FOR CHEMICAL CARCINOGEN INDUCED DNA DAMAGE

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OBJECTIVE: The initiation of carcinogenesis would appear to involve the chemical binding to DNA. This binding usually results in alkaline sensitive sites and/or breaks that can be detected by the alkaline elution and unwinding techniques.

APPROACH: The project will develop these DNA damage can be used as a molecular marker for carcinogenesis and in the study of the dose dependency and pharmacokinetic parameters chemical carcinogenesis.

111. REPAIR OF CARCINOGEN-INDUCED DNA DAMAGE IN HUMAN CELLS - THE ROLE OF ADP RIBOSYLATION

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The overall objective of our work is to understand more about the way in which human cells repair DNA damage induced by carcinogens. The aim of this research program is to investigate the possible relationship between ADP ribosylation and DNA damage and repair. We plan to evaluate the role of ADP ribosylation in DNA repair in human cells damaged with direct-acting chemical carcinogens and ultraviolet radiation. Chemical carcinogens will include: 'short patch', X ray-mimetic agents (e.g., methylnitrosourea (MNU) and methylnitronitrosoguanidine (MNNG)); and 'long patch', UV-mimetic agents (e.g., N-acetoxy-2-acetylaminofluorene (NA-AAF) and 7-bromomethylbenz (a)-anthracene (BMBA)). If stimulation of ADP ribosylation by long patch agents is confirmed, repair deficient xeroderma pigmentosum cells damaged with these agents will be used to determine whether the stimulation of ADP ribosylation is the result of DNA damage alone or is part of the DNA repair process. Using polyacrylamide gel electrophoresis we will examine ADP ribosylation of nuclear proteins during repair with special emphasis on nucleosome structure and histones H1 and H2B.

112. DEVELOPMENT OF A TISSUE-SELECTIVE IN VIVO BIOASSAY FOR ENVIRONMENTAL AGENTS PRODUCING DNA DAMAGE

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OBJECTIVE: Integrate selected in vitro assay procedures into the animal model system in order to develop a rapid, inexpensive in vivo bioassay system that will permit a quantitative, tissue specific prediction of the carcinogenic potential of environmental agents alone and in combination with one another.

APPROACH: Micropunch tissue biopsies will be removed, the cellular matrix dispersed, and DNA assayed by use of sucrose gradients or agarose gels. The detection of DNA following separation by molecular weight will involve the use of a spectrofluorometric assay permitting analysis of DNA concentrations in the nanogram range.

113. PATHOLOGY OF REPAIR OF CARCINOGENIC DNA DAMAGE

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The enzymatic initiation of DNA repair of specific forms of damage - 5,6-dihydrodihydroxythymine, pyrimidine dimers and interstrand cross-links - will be investigated. Substrate supercoiled DNA containing only one type of such damage will be prepared. Repair endonucleases will be assayed by measuring the conversion of the damaged supercoiled DNA to the nicked form. These activities in normal cells will be compared to those in xeroderma pigmentosum and Fanconi's anemia, genetically transmitted diseases with high incidences of cancer. Deficiencies will be explored by standard biochemical techniques, including purification of these enzyme activities. The results will elucidate the mechanisms of initiation of excision repair of such DNA damage in normal cells and its defects in human diseases that lead to the development of cancer.

BIBLIOGRAPHIC REFERENCES: Duker, N.J. and Grant, C.L., Alterations in the Levels of Deoxyuridine Triphosphatase, Uracil-DNA Glycosylate and AP Endonuclease During the Cell Cycle, Experimental Cell Research, 125: 493-497, 1980. Duker, N.J. and Davies, W.A., Photochemistry of Uracil-Containing DNA, Federation Proceedings, 39, 1937 (abstract), 1980.

114. STUDIES OF DNA DAMAGE AND REPAIR BY ANTITUMOR AGENTS

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Current efforts in this laboratory are directed towards the molecular mechanism of action of three classes of anti-cancer agents. The mechanism by which intercalating agents such as adriamycin and actinomycin-D produce strand breaks in DNA is being investigated. We have found both single strand breaks and double strand breaks in mammalian cells following exposure to these drugs and we are attempting to determine what cellular enzyme is responsible for these breaks. We have isolated a topoisomerase which we believe may be responsible for this lesion and we are attempting to characterize this enzyme further.

In addition, we are looking for evidence that hexamethylmelamine and pentamethylmelamine act by damaging DNA. We have already shown that single strand breaks and DNA-DNA crosslinks do not result following treatment of mammalian cells

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with these drugs in cytocidal conditions. We are now looking for evidence of monofunctional adduct formation.

Finally, correlation between DNA crosslink formation, DNA synthesis inhibition, and cell cycle traverse perturbations following treatment of mammalian cells with bifunctional alkylating agents is being pursued. We are combining the techniques of alkaline elution, thymidine incorporation, and flow microfluorometry to elucidate this problem.

115. REPARABILITY OF CHEMICAL CARCINOGENIC DAMAGE TO DNA

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Human uracil-N glycosylase is purified from normal full term placentas. The purification follows standard ion exchange chromatographic techniques followed by isoelectric focusing. Separation of the accompanying endonuclease activity is being effected through the use of chaotropic agents and selective denaturation techniques.

Modified thymine derivatives in DNA arise from oxidation and/or free radical reactions and may be the substrate for the endonucleolytic activities present in both pro and eukaryotic cells directed against oxidized and irradiated DNA. We have developed a rapid purification of 5,6-dihydroxydihydrothymine (which is thought to be the principal modification of DNA after oxidation or irradiation), and will use this as a marker to determine the specificity of enzymatic activity directed against such modified DNA.

BIBLIOGRAPHIC REFERENCES: On the nature of the human endonuclease activity directed against ultra-violet irradiated DNA. Teebor, G., Goldstein, M., Frenkel, K., Duker, N., and Brent, T. in DNA Repair Mechanisms, P.C. Hanawalt and E.C. Friedberg, Eds., Academic Press, 1978.

116. VISCOELASTIC ANALYSIS OF BRAIN AND TUMOR DNA

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The summary for this project is under entry no. 84.

117. REPAIR SYNTHESIS OF DNA IN PRECANCEROUS RAT LIVER

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The objective of this research project is to ascertain the capacity for repair of specific types of carcinogen-induced DNA-base damage in the nuclei of hepatocyte and cell populations of precancerous rat liver. This may lead to an identification of those situations in which the possibility of aberrant gene expression becomes increasingly likely. Precancerous liver will be obtained by maintaining rats on diets containing either 2-acetylaminofluorene or 4-dimethylaminoazobenzene. The effect of the duration of carcinogen ingestion on the degree of covalent binding to, and the rate and extent of removal of carcinogen from, the DNA of the two hepatic cell populations will be determined. DNA extracted from the nucleus fractions of precancerous liver will be enzymatically digested to nucleosides which will be separated, by employing high pressure liquid chromatography, to discern specific reaction products of the carcinogen with DNA bases. An attempt will be made to determine whether the capacity to repair specific types of hepatic DNA-base damage is altered during the continued feeding of hepatic carcinogens. The capacity of DNA from precancerous liver to serve as a template for RNA synthesis will be measured and used as a functional measure of DNA repair. The activities of several enzymes involved in the DNA repair process, and the degree of single strand interruptions in DNA, will be determined in precancerous liver as a function of time on a carcinogenic diet. Novikoff hepatoma cells grown in suspension culture will be employed for studies on the fidelity of the DNA repair process, as assessed by changes in mutation frequency.

BIBLIOGRAPHIC REFERENCES: Schwartz, Edward L., and Goodman, Jay I.: Preferential carcinogen modification of specific hepatic chromatin fractions. Proceedings of the American Association for Cancer Research 19: 34 (1978). Schwartz, Edward L. and Goodman, Jay I.: Studies on carcinogen-chromatin interactions. Federation Proceedings 37: 749 (1978).

118. STUDIES OF DNA CHANGE INDUCED BY PANCREATIC CARCINOGENS

Longnecker, D. S., Yager, J. D., Curphey, T. J., Unger, J., Dartmouth College, Dartmouth Medical School, Dept. of Pathology, *P.O. Box 833, Hanover, New Hampshire*, 03755, United States of America

The objective of this project is evaluation and comparison of damage of pancreatic DNA induced by several experimental carcinogens in rats. As specific parameters of damage are defined in the course of working with DNA from whole pancreas, studies will be extended to compare DNA damage in acinar cells and duct cells, and to compare DNA damage in pancreas with that in several other tissues. The compounds which will be evaluated include azaserine, N-gamma-(N-methyl-N-nitrosocarbamoyl)-L-2,4 diaminobutyric acid, N-nitrosobis (hydroxypropyl) amine and agaritine. Technical approaches include synthesis of radioactive carcinogens, DNA alkylation studies (carcinogen binding), and identification of specific abnormal nucleosides.

119. ERROR-PRONE DNA REPAIR IN HEPATOCARCINOGENESIS

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The main objective of the work proposed is to study the role of post-replication DNA repair processes during the initiation of hepatocarcinogenesis in vivo. Rats are treated with N-hydroxy-2-acetylaminofluorene at various doses and times relative to enhanced liver DNA synthesis caused by surgical partial hepatectomy. The doses and times will be sufficient to cause varying degrees of short-term inhibition of regenerative DNA synthesis followed by recovery and are being experimentally determined. Post-replication DNA repair processes occurring during the recovery of DNA synthesis from carcinogen treatment will be examined using several techniques. The presence or absence of error-prone DNA synthesis/repair processes will be determined in two ways. One way involves the in vitro determination of the fidelity with which crude DNA polymerase fractions isolated from liver nuclei at appropriate times carry out DNA synthesis. The other way involves the use of cultured hepatocytes obtained from the livers of animals treated in vivo. Such hepatocytes will be infected with UV-irradiated herpes virus to determine the effect of the prior in vivo treatment on hepatocyte mediated herpes reactivation and mutagenesis. Post-replication repair will also be measured in vitro in cultured hepatocytes isolated from regenerating liver and thus still carrying out DNA synthesis in culture. Following carcinogen treatment, alkaline sucrose gradient centrifugation or alkaline elution will be used to detect discontinuities and their repair in newly synthesized DNA. The extent of occurrence of these various post-replication processes will be correlated with the amount of excision repair occuring between carcinogen treatment and induced DNA synthesis and with the ability of the treatment protocols to induce preneoplastic nodules in liver.

120. THE ROLE OF DNA REPAIR IN THE GROWTH AND THERAPEUTIC RESPONSES OF SOLID TUMORS

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DNA repair has been shown to play a role in carcinogenesis since humans with genetic defects in repair are known to be cancer-prone. The major type of repair which has been studied in human is excision repair. Some cancer-prone individuals have a defect in a type of repair, known as post-replication repair (PRR) in which damage to DNA is by-passed during the course of DNA replication. The role of PRR in chemotherapy and in cancer growth and dissemination has not been studied extensively. PRR is considered to be an error-prone process since replication occurs on damaged templates, thus cells which are proficient in PRR should be more mutable. Increased mutability could result in a greater range of responses of tumor cells to therapeutic agents and with higher frequencies of resistant cells. Ability to by-pass DNA damage could also lead to enhanced

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resistance to therapy. We have examined the ability of mouse melanoma cells and mammary carcinoma cells to perform PRR and have found that this is enhanced relative to the ability of a normal mouse lung fibroblast line to perform PRR. The mouse system is useful for PRR studies as the level of excision repair is very low in these cells, so results do not need to be corrected for excision repair. We are now investigating the role of PRR in the response of tumor cells to chemotherapeutic agents and the ability of chemotherapeutic agents to enhance or inhibit PRR. The role of PRR in the response of human tumor cells to chemotherapeutic agents and to radiation therapy will be investigated. The level of PRR in tumor cells will be determined in order to correlate therapeutic response determined by patient follow-up with PRR.

121. DNA REPAIR DEFICIENCY AND INCREASED PROBABILITY OF CANCER IN CERTAIN HUMAN GENETIC DISEASES

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The summary for this project is under entry no. 81.

122. THE ROLE OF DNA REPAIR IN THE THERAPEUTIC RESPONSES OF MELANOMA

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The summary for this project is under entry no. 80.

123. DNA REPAIR IN RELATION TO THE CELL CYCLE

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The summary for this project is under entry no. 75.

124. DNA REPAIR AND RECOVERY IN THE MAMMALIAN CELL CYCLE

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The summary for this project is under entry no. 78.

125. POSTREPLICATION REPAIR IN CULTURED MAMMALIAN CELLS

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The objective of the proposal is to determine the molecular events involved in replication of a chemical carcinogen damaged DNA template in cultured mammalian cells treated with the proposed ultimate carcinogenic form of benzo(a)pyrene, 7,8 dihydroxy 9,10-epoxy-7,8,9,10-tetrahydrobenzo(a) pyrene, (B(a)P-diol-epoxide). The proposed studies make use of primary cultures of mouse epidermal cells and Vero cells (an African green monkey kidney cell line). Four specific objectives include 1) to continue to develop evidence for 'gapped DNA synthesis' in epidermal cells using DNA fiber autoradiography in order to study DNA fork progression and replicon function, 2) to determine if B(a)P-diol-epoxide DNA adducts in Vero cells act as absolute blocks to DNA replication and if not to determine the mechanism by which the whole genome is replicated, 3) to determine if B(a)P-diol-epoxide treatment of epidermal cells or Vero cells results in host cell reactivation of either UV-irradiated or B(a)P-diol-epoxide treated herpes simplex virus and whether reactivation is mutagenic to the virus and inducible, 4) to determine at the molecular level whether there is an enhanced rate of DNA elongation which might be related to B(a)P-diol-epoxide induced reactivation of the herpes simplex virus.

BIBLIOGRAPHIC REFERENCES: Bowden, G.T., and Fusenig, N.E., Caffeine Inhibition of Postreplication Repair of UV-Damaged DNA in Mouse Epidermal Cells. Chem. Biol. Interact. (in press), 1980.

126. IN VITRO STUDIES IN CHEMICAL HEPATOCARCINOGENESIS

Michalopoulos, G., Duke University, School of Medicine, Dept. of Pathology, *Box 3711, Durham, North Carolina*, 27710, United States of America

Primary cultures of hepatocytes on collagen membranes will be used as a model for in vitro studies in chemical hepatocarcinogenesis. The objective is to study the interaction of chemical carcinogens and hepatocytes in terms of repair of DNA damage and neoplastic transformation of hepatocytes. DNA repair will be assessed as unscheduled DNA synthesis, repair of single or double strand DNA breaks and removal of carcinogens bound to the hepatocellular DNA. Correlations between carcinogenicity and patterns of repair of DNA damage elicited by chemical carcinogens will be attempted. The effects of hormones and other biologically active compounds on these processes will be analyzed. Hepatocytes from adult rat livers (normal and preneoplastic) and neonate rat livers will be used. Primary cultures of hepatocytes from adult rat normal and regenerating rat liver will be exposed to chemical carcinogens in order to study the production of 'altered' hepatocytes. This process, already shown to occur in vitro with the procarcinogen AAF, will be studied with other chemical carcinogens. The effect of hormones and other biologically active compounds in this process will be analyzed. The neoplastic potential of the altered hepatocytes will be evaluated with in vitro and in vivo techniques (growth in soft agar, formation of tumors in syngeneic animals, etc). These experiments will help to increase understanding of the factors that determine the outcome of interaction between carcinogens and hepatocytes and the growth of initiated hepatocytes at the early stages of hepatic neoplasia.

BIBLIOGRAPHIC REFERENCES: G. Michalopoulos, C. Biles, F. Russell: Studies in Hepatocarcinogenesis Using Primary Cultures of hepatocytes. In: The Liver: Quantitative Aspects of Structure and Function, Vol III, R. Preissig, J. Burcher and G. Baumgartner (Editors), Editio Cantor, Switzerland, pp. 135-146, 1978. G. Michalopoulos, C. Biles and F. Russell: Primary Cultures of Hepatocytes on Confluent Human Fibroblasts. In Vitro 15:796-806, 1979.

127. THE ROLE OF DNA REPAIR DAMAGE AND ITS REPAIR MECHANISMS IN IN VITRO CHEMICAL TRANSFORMATION

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The DNA damages induced by carcinogens and their repair mechanisms are generally believed to be involved in cell transformation processes, but their direct roles are poorly understood at the molecular level. Variants subcloned from BALB/3T3 A31 cone 1 have been found to exhibit different sensitivity to chemical-induced transformation. The purpose of this work is to determine whether the excision repair of benzo(a)pyrene (BP)-damaged DNA (i.e. BP-deoxyguanosine) of these cells is responsible for their different response to chemicals. With the three classes of variants studied, it has been found that all variants excised carcinogen-damaged DNA with extremely low efficiency. They are very much like the cells obtained from Xeroderma Pigmentosum (XP) patients with respect to repair deficiency.

Two immunological methods have been explored to assay the minute amount of BP-DNA formed in human KD and XP cells.

128. CHEMICAL CARCINOGENESIS (WITH EMPHASIS ON N-NITROSO COMPOUNDS)

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OBJECTIVE: To establish the mode of action of carcinogenic N-nitroso compounds.

APPROACH: On the basis of a detailed knowledge of DNA reaction sites, following treatment with N-nitroso compounds in vivo, the relative stability and miscoding potential of particular lesions are being studied. The emphasis has been placed on extending our knowledge of the DNA repair system

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for O6-alkylguanines in which both acute and chronic administration of different N-nitroso compounds have been examined in rats and Syrian and Chinese hamsters. The effect of pretreatment with hepatocarcinogens, structurally unrelated to N-nitroso compound, is also being examined. Using an in vitro system it has been shown that the presence of O4-methylthymine in synthetic polynucleotides will cause miscoding with a DNA polymerase. Although this is a minor product, in vivo studies are being made to assess its significance in the carcinogenic process.

129. EFFECTS OF PESTICIDES ON DNA SYNTHESIS, REPAIR AND CELLULAR TRANSFORMATION IN MAMMALIAN CELLS CULTURED IN VITRO (ABBREV)

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OBJECTIVE: To study the effects exerted by pesticides utilized in agriculture on mammalian and human cells cultured in vitro. Particular attention is devoted to the interferences of these chemicals with cellular informative structures.

APPROACH: The parameters considered are: 1) the inhibition of scheduled DNA synthesis exerted by pesticides; 2) the capability of these chemicals to induce damages in DNA molecules measured by tritiated thymidine uptake by cells cultured in the presence of hydroxyurea; 3) their capability of depressing cellular repair processes following damages to DNA exerted by physical agents (i.e. UV or gamma rays); 4) morphological changes and/or transformations of cells cultured with or without pesticides and ultimate carcinogens (i.e., loss of contract inhibition).

PROGRESS: The study of 18 pesticides as agents damaging to human lymphocyte DNA synthesis has shown that: 1) some of them elicit low reparative DNA synthesis; 2) some of them exert an inhibition of DNA reparative synthesis after UV irradiation; 3) lack of correlation between the effects exerted on scheduled and unscheduled DNA synthesis occurs.

130. REPAIR OF PLATINUM-INDUCED DNA DAMAGE

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The objective of this project is to study the role of DNA repair processes in determining the response of tumor cells and normal tissues to several platinum coordination complexes. Since DNA is probably the molecular target of the platinum antitumor drugs, an understanding of the mechanisms whereby cells handle platinum-induced damage in DNA might lead to improvements in chemotherapy with these agents. The dose-survival curves of several rodent neoplasms and normal hematopoietic cells will be measured for several platinum compounds using in vivo and in vitro colony formation assays, and the effects of dose fractionation will be determined. Then, excision of platinum residues from DNA over time will be followed in normal cells and in sensitive and resistant tumors after single and split dose regimens. Additionally, post-replication repair processes which lead to synthesis of full-size DNA on a template containing platinum lesions will be studied in several tumor cell lines with single and split dose regimens. The possible induction of such processes will be considered.

131. DNA REPAIR/REPLICATION IN CHEMICAL CARCINOGENESIS

Sarma, D. S., Rajalakshmi, S., Columbano, A., Rao, P. M., University of Toronto, Dept. of Pathology, *Medical Sciences Bldg., Toronto, Ontario,* M5S 1A8, Canada

Results obtained indicated that cell proliferation and, more specifically, replication of carcinogen-damaged DNA prior to the repair of a critical lesion or lesions appear to be an essential step in the induction of initiated hepatocytes. Initiated hepatocytes were monitored by selective in vivo proliferation of these cells into islands of putative preneoplastic hepatocytes positive for gamma-glutamyl transpeptidase (Nature 275:60-62, 1978).

Experiments are in progress to determine the critical lesions in liver DNA, induced by carcinogens such as N-methyl-N-nitrosourea, 1,2-dimethylhydrazine and diethylnitrosamine, responsible for the development of preneoplastic hepatocyte populations. Experiments are also in progress to determine whether replication of carcinogen-damaged DNA, prior to the repair of the critical lesion, is an obligatory step for the induction of not only preneoplastic but also neoplastic hepatocytes.

Experiments designed to determine the carcinogen-induced lesions in DNA that permit in vivo replication revealed that N-7-methylguanine, O6-methylguanine, N-3-methyladenine and possibly phosphotriesters induced by dimethylnitrosamine in rat liver DNA permit in vivo replication. Currently experiments are in progress to determine the nature and in vivo fate of such carcinogen-modified replicated DNA.

BIBLIOGRAPHIC REFERENCES: Rao, Prema M., Rajalakshmi, S. and Sarma, D.S.R. Carcinogen induced alteration in gene packing and its possible significance in carcinogenesis. In Chromatin Structure and Function. (Nocolini, C., ed.) Plenum Publications, New York (1979), in press. Sarma, D.S.R., Rajalakshmi, S., Udupa, R.S., Farber, E. and Rao, Prema M. Factors influencing DNA damage and its repair with cellular implications for toxicology. In Reactive Metabolites and Their Implications for Toxicology. 7th International Congress of Pharmacology. Pergamon Press, New York. (1979), in press.

132. EFFECTS OF BIFUNCTIONAL ALKYLATING AGENTS ON DNA- AND RNA-SYNTHESIS IN HUMAN MYELOMA AND MELANOMA CELLS

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OBJECTIVE: To study factors in human tumor cells responsible for resistance and sensitivity to alkylating agents.

APPROACH: Human myeloma and melanoma cells are collected for short-term culture. The cells are damaged with bifunctional alkylating agents and the effects on replicative DNA synthesis, RNA synthesis and DNA repair synthesis are recorded.

PROGRESS: The effect of melphalan on DNA replication and transcription in human melanoma cell populations is less than the effect on human phytohemagglutinin stimulated lymphocytes. Melphalan induces low levels of DNA repair synthesis in human melanoma cells. The fact that UV-light gives rise to high levels of DNA repair synthesis in the same cells indicates that melphalan gives rise to low levels of DNA damage in human melanoma cells. Human myeloma cells differ in capacity for DNA repair synthesis depending on the degree of differentiation. The material is extended, the aim being to study clinically sensitive and resistant myeloma cell populations regarding melphalan induced DNA repair synthesis. Cytofluorometric measurements of human melanoma cell populations indicate that the majority of tumor cell populations contain aneuploid cells, and that most cells have a hyperdiploid DNA value. There is a great variation in DNA synthesizing cells in different melanoma tumors. These data will be related to the clinical response to alkylating agents.

133. REPAIR OF COVALENT ADDUCTS FORMED BETWEEN BENZO(A)PYRENE (B(A)P) AND DNA BY MOUSE EMBRYO FIBROBLASTS

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Since most mammalian cells have evolved mechanisms for removing damage from their DNA we are investigating whether mouse fibroblast cells in tissue culture can excise B(a)P adducts from their DNA. Cells, labelled with 14C-thymidine while growing to confluence, are exposed to 3H-B(a)P for six hours; they are then maintained, for up to six days, in a resting state in medium containing 0.5 percent serum. When the DNA is extracted and degraded to nucleosides, the nucleosides containing 3H-B(a)P adducts can be separated on LH20-Sephadex columns, and the rate at which they are removed can be measured. Although approximately 60 percent of the adducts are removed within 72 hours, the remaining 40 percent are much more resistant to excision and these adducts are not removed when the cells double in number. We are attempting to determine the distribution of the repair-resistant adducts in the mouse DNA by the methods described in a previous report (ICRF Scientific Report 1978 -report 214) and to isolate from mouse embryos by methods already developed, (ICRF Scientific Report 1977 - reports 197 and 198) the proteins involved in the excision of the

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adducts. Similar methods are being used to study the binding to, and excision from, mouse embryo fibroblasts of compounds of the cyclopenta(a) phenanthrene series synthesized in the Chemical Laboratory. We are collaborating with the Cellular Pathology Laboratory to use baby mouse kidney cells grown in tissue culture to provide primary epithelial cells on a scale large enough to permit us to use our biochemical techniques.

134. SYNTHESIS OF INTERCALATING DRUGS AS INHIBITORS OF DNA REPAIR MECHANISMS

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OBJECTIVE: Intercalation in DNA is an important feature of the mode of action of many anti-cancer drugs. All such drugs are essentially flat. It is proposed that drugs of rigid structure incorporating a bridging unit will, because of slight deviation from planarity, still intercalate in DNA but because of non-planarity, lead to larger unwinding angles of the DNA. The project concerns the synthesis of suitable bridged compounds, a study of their interaction with DNA and the testing of their biological activity.

APPROACH: Based on readily available bicyclo (3,3,1) nonadiene diones, the synthesis of carbocyclic bridged annulenes and heterocyclic bridged annulenes is planned. In particular, heterocyclic skeletons incorporating bridged 1,5-benzodiazepines will be synthesized. Bridged compounds related to aminoquinolines, to diacridines and to styrylquinolines are to be investigated. Following synthesis and biological testing, detailed studies of the more promising compounds with DNA will be made.

135. GENETIC STUDIES OF CELLULAR RESISTANCE TO PHYSICAL AND CHEMICAL CYTOTOXIC AGENTS

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The summary for this project is under entry no. 86.

136. DNA REPAIR AFTER POLYCYCLIC HYDROCARBON ADMINISTRATION

Bresnick, E., Eastman, A., University of Vermont & State Agricultural College, School of Medicine, Dept. of Biochemistry, *85 S. Prospect St., Burlington, Vermont,* 05401, United States of America

The objective of this line of research is to understand some of the factors which are responsible for the removal of deoxyribonucleoside-adducts from DNA. In this regard, we will determine the effects of benzo(a)pyrene (BP) upon the alkaline elution profile of a cloned hamster tracheal cell line. We will determine the nature of the adducts; their biological stability as altered by vitamin A analogs and chronic administration of BP; their occurrence in micrococcal nuclease sensitive- and -resistant portions of the tracheal chromatin. The effects of asbestos (crocidolite) upon these parameters will be measured and the ability of the vitamin A analogs to prevent any asbestos effects will be pursued. Finally, we will continue studies on the purification of the enzyme system present in rat liver which is responsible for the removal of O6-methylguanine from methylated DNA (after methylnitrourea). We will initiate studies in the repair enzymes involved in the removal of deoxyguanosine-BP and deoxyadenosine-BT adducts from DNA alkylated with the diol epoxides of BP. We will employ liver and tracheal extracts.

BIBLIOGRAPHIC REFERENCES: Eastman, A. and Bresnick, E.: Persistent binding of 3-methylcholanthrene to mouse lung DNA and its correlation with susceptibility to pulmonary neoplasia. Cancer Research 39:2400-2405 (1979). Eastman, A., and Bresnick, E.: Metabolism and DNA binding of 3-methylcholanthrene. Cancer Research 39:4316-4321 (1979).

137. DNA REPAIR AND CHEMICAL CARCINOGENESIS

Cox, R., University of Tennessee. Center for the Health Sciences, School of Medicine, Dept. of Biochemistry, *62 S. Dunlap St., Memphis, Tennessee,* 38103, United States of America

The proposed research is concerned with the initiating events of chemical carcinogenesis. Major emphasis will be on the interaction of carcinogens with DNA and the consequence (damage and repair) of this reaction as it relates to the integrity of the cell. Damage and repair will be studied with alkaline sucrose gradients, BND-cellulose, and determination of the alkylated products. A single initiating dose of carcinogens such as methylnitrosourea will be used to examine damage and repair. The effect of carcinogens on DNA synthesis (repair and replication) and loss of alkylated products, including phosphotriesters, will be studied.

138. CHEMICAL CARCINOGENESIS AND DNA REPAIR

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Objectives are to isolate and purify enzymes which recognize alkylated DNA bases and to define the extent of their action on DNA of subunits of chromatin. Purification of a procaryotic and a eucaryotic 3-methyladenine glycosylase continues and search for a procaryotic activity which recognizes 0-6 methylguanine. Quantitative and qualitative aspects of alkylation in vitro of nucleosomal DNA and the recognition of the alkylated bases by enzymes is under investigation. Repair of DNA treated with Beta-propiolactone is being examined.

139. REGULATION OF DNA REPAIR IN CHEMICAL CARCINOGENESIS

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Human cells contain singular DNA repair pathways to excise critical lesions from DNA. In individuals with clinical syndromes predisposing to malignancy, the capacity to excise these lesions is impaired. This research proposal is designed to investigate the ability of human cells to actively regulate DNA repair pathways as they traverse the cell cycle. In particular, we seek to determine whether normal human cells modulate the pathway for nucleotide excision repair or the pathway for base excision repair as they are synchronously stimulated to proliferate. Further, we seek to examine whether the regulation of singular DNA repair pathways are defective in cells from individuals with clinical syndromes predisposing to malignancy. The objectives of this proposal are: 1) to characterize the induction of nucleotide excision repair and of base excision repair in synchronous populations of WI-38 cells stimulated to enter the cell cycle. 2) to determine the sequence with which normal human cells enhance the potential for DNA repair with respect to the induction of DNA replication. 3) to determine whether the failure of repair defective human cells to excise critical DNA lesions can be causally related to their inability to regulate those DNA repair pathways responsible for the excision of those lesions.

140. DNA REPAIR MECHANISMS IN CARCINOGENESIS

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The summary for this project is under entry no. 58.

141. LIGHT-INDUCED MODIFICATION OF DNA WITH COUMARYL COMPOUNDS

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Coumaryl compounds are synthesized naturally by plants and fungi and are common in our environment. Psoralens and aflatoxins, particular kinds of coumaryl compounds, contain a light-absorbing structure called coumarin, which is responsible for the photochemical reactivity of these compounds. Psoralens are known to cause skin cancer in the presence of near ultraviolet light (UV), while aflatoxins (AF) are known to be a potent liver carcinogen as well as inducers of skin tumors in white mice. The toxic actions of these coumaryl compounds are apparently a result of their ability to modify the genetic material, deoxyribonucleic acid (DNA), which is present in every living cell. This award should elucidate the chemical nature of DNA mofification by psoralens and AF. Specifically, the researchers will determine which component bases on DNA are photomodified by cancer-inducing psoralens and AF's and then evaluate the relative importance of DNA modification by psoralen and its derivatives. In addition, the utility of these newly-synthesized psoralen-labelled and AF-labelled DNA's as new research tools in molecular biology and cancer research will be ascertained.

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142. DISRUPTION IN NUCLEIC ACID DUPLEXES CAUSED BY THE INCORPORATION OF O6-ALKYLATED GUANINE RESIDUES AS PROBED BY PROTON NMR

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OBJECTIVE: Modified bases, such as O6-methyl-guanosine, when incorporated into DNA duplexes cause disruption in the duplexes and result in mutagenesis and cancer if not removed by DNA repair enzymes. The precise perturbations caused by these bases remain obscure. A more detailed knowledge of the alterations in the overall duplex shape caused by these bases should lead to a better understanding of why DNA repair enzymes are not 100% effective in excising these bases and ultimately of the molecular basis of disease and potential strategies for combatting it.

APPROACH: We wish to assemble single strand sequences containing major bases which have been alkylated (1) at different positions, and (2) with different alkyl groups. It is intended to duplex these strands with sequencee of bases which are nearly complementary and anneal them by temperature lowering. Proton magnetic resonance spectroscopy (1H NMR) will be used during the annealing process to observe the behavior of the modified bases as well as the behavior of the nearest and next-nearest neighboring bases. These studies will lead to stability (delta H) information, as judged from melting temperatures (Tms), together with spatial information on the orientation and separation of the bases which will be derived from the chemical shifts of protons on the individual bases and the ribose H-1-1 protons. It is proposed to examine the incorporation of O6-alkyl guanosine within the self complementary parent duplex CAUG:CAUG and carry out mixing experiments with nearly complementary sequences as shown in I-III: Duplex I (parallel strands) - CAGUG:GUCAC; Duplex II (parallel strands) - CAG(star)UG:GUCAC; Duplex III (parallel strands) - CAG(star)UG:CUUAC. G(star) equals O6R-guanosine; R equals CH3, -CH2CH3, -CH(CH3)2.

Duplex I represents the perfect control duplex. These experiments will show how duplex stabilities change with R and how the G(star) residue changes its geometric position, relative to duplex I, as the alkyl group increases in size. Alteration of the spatial relationship of the adjacent and opposite bases can also be followed from the behavior of protons on the A, U and C residues. (Text Abridged.)

143. REACTION OF CARCINOGENS AND CELL EXTRACTS WITH RF-DNA

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A large proportion of human cancer is a consequence of the action of external carcinogenic chemicals. This carcinogenicity may be a consequence of the alteration of cellular DNA. One possible type of alteration of DNA that could be of significance is a modified base or nucleotide in one of the duplex strands which might lead to errors in DNA duplication and information readout. In the proposed experiments we will determine the potential of two series of chemical carcinogens for producing nucleotide damage in DNA in vitro. As a sensitive test system we will utilize phi X174 replicative form (RF)I DNA, a closed circular, supercoiled, double-stranded DNA of MW 3.4 times 10 to the 6th power. With this DNA, as few as 1 in 10 to the 5th power altered nucleotides can be easily detected under neutral conditions; this amounts to 1 per 10 molecules. The carcinogens to be used are: 1) Acetylaminofluorene (AAF) and its derivatives, N-hydroxy-acetylaminofluorene (HO-AAF), and N-acetoxyacetylaminofluorene (AcO-AAF) and 2) benz(a)anthracene (BA) and dibenz(a,h)anthracene (DBA) and several dihydrodiol and epoxide derivatives. Particular attention will be paid to the suspected 'ultimate' carcinogenic form of these, AcO-AAF and the K-region epoxides of BA and DBA. Following reaction of the chemical with the RFI DNA at physiological pH, the unbound carcinogen will be removed by column chromatography and the RF-DNA directly assayed for nucleotide damage and strand breaks by sucrose gradient sedimentation, or further treated by alkali, near-UV light or exposure to extracts of human cells, followed by sucrose gradient sedimentation. The gradients will be fractionated, the labeled peaks located and quantitated by liquid scintillation counting and the biological activity of the fractions assayed by the ability of the RF-DNA to infect calcium-treated host bacteria. Host bacterial cells from a series of well defined radiation repair mutants will be used to determine the ability of specific repair systems to act on carcinogen-treated DNA, and to detect the presence of unrepairable nucleotide damage which may be responsible for the carcinogenesis of human cells.

144. STRUCTURAL AND FUNCTIONAL MODIFICATION OF DNA BY BENZO(A)PYRENE METABOLITES

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The topic of the present study is the establishment of a highly sensitive and accurate method for detection of the structural modification of DNA caused by covalent and non-covalent interactions with benzo(a)-pyrene (BP) metabolites.

The effects of carcinogens bound to DNA on the functions of that DNA were studied by assaying replication, mutation, and repair of the DNA using both an in vitro replication system and a unique mutation assay system developed in this laboratory.

Several different clones of plasmid DNA which were mutated by a small number of BP metabolites are selected. Their transformability, restriction endonuclease map, and DNA base sequence are currently being studied in order to determine the location and chemical nature of DNA modification.

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145. ACTIVATION AND REACTIVITY OF NITROHETEROCYCLES

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Humans are exposed to nitroheterocyclic compounds through extensive use in medicine and industry; these chemicals are metabolically 'activated' to toxic, DNA damaging and, in many cases, tumorigenic agents. We will attempt to determine the relationship between drug 'activation,' DNA damage, mutagenicity and transforming ability of selected nitroheterocycles. Specifically, we will determine whether the electronegativity (reactivity of these chemicals) as reported in the literature, is correlated with their ability to damage DNA, as measured by hydroxylapatite chromatography, their mutagenicity in bacterial tester strains, or their ability to transform cultured C3H/10T1/2 mammalian cells. In addition, we intend to determine whether the amount of nitrofuran-induced DNA damage or its rate of repair differs in specific target organs for tumor production, and further, whether this damage is due solely to metabolic activation in the target tissue or to tranfer of 'activated' intermediates of nitrofuran metabolism.

146. EXCISION-REPAIR OF BENZO(A)PYRENE-MODIFIED DNA

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Conditions were determined for achieving the highest level of binding of metabolized B(a)P to double stranded salmon sperm DNA in the presence of microsomal metabolizing system. The binding level was up to 50 fold higher than that reported in the literature. Thus, an appropriate substrate DNA has been prepared for the experiments leading to the elucidation of the nature of the putative (∃Pase) activity.

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A rapid method of purification of 5, 6-dihydroxydihydrothymine (thymine glycol, TG) was developed. TG is used as a marker to determine the nature of modified thymine residues in DNA and to determine the specificity of enzymatic activity directed against such modified DNA, including benzo(a)pyrene modified DNA.

147. DNA REPAIR AND ITS RELATIONSHIP TO CARCINOGENESIS

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These studies involve the isolation, purification and characterization of enzymes involved in the repair of base damage to DNA. Such enzyme activities are isolated from bacteriophage T4-infected E. coli, uninfected E. coli and the yeast S. cerevisiae.

BIBLIOGRAPHIC REFERENCES: Cone, R. and Friedberg, E.C. Purification of Uracil-DNA Glycosylase from Bacillus subtilis. In DNA Repair: A Laboratory Mannual of Research Procedures, Eds. E.C. Friedberg and P.C. Hanawalt, Marcel Dekker, Inc., New York, N.Y., Vol. I, Part A, p. 253, 1981.

148. INTERACTION OF CARCINOGENS WITH DNA - REPAIR OF LESIONS

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We have investigated the interaction of carcinogens with DNA of human fibroblasts and the effect of DNA repair on this interaction. Diploid human cells offer a unique advantage because of their high rates of excision repair and the availability of repair deficient XP strains. We have demonstrated that if repair proficient cells are prevented from replicating, but allowed to excise, they can eliminate potentially lethal and mutagenic effects of carcinogens. We have developed quantitative methods to measure the rate of repair replication and the removal of specific carcinogen residues covalently bound to DNA. The rate of excision determines the ultimate cytotoxic effect of carcinogens and the number of unexcised lesions remaining at the time semi-conservative DNA replication takes place ultimately determines the mutagenic effect of the damage. Because genetically altered cell characteristics may be responsible for the initiation of malignant transformation, we propose to investigate: 1) The kinetics of excision repair induced by different carcinogens, the number and kind of adducts removed, the nature of the excised region, and whether these adducts cause significant distortion of the DNA in order to correlate these with the biological consequences of carcinogen treatment; 2) The critical relationship between the rate of excision repair, the length of cell cycle, and the biological effects of carcinogens; 3) The postreplication repair mechanisms used by human cells to cope with unexcised damage; 4) Whether the carcinogenic potency of a series of structurally-related polycyclic hydrocarbons is correlated with differences in rates of metabolism, rates of binding to DNA, rates of excision repair, or intrinsic mutagenic potency of particular DNA adducts.

BIBLIOGRAPHIC REFERENCES: R.H. Heflich, R.M. Hazard, L. Lommel, J.P. Scribner, V.M. Maher, and J.J. McCormick. A comparison of the DNA binding, cytotoxicity and repair synthesis induced in human fibroblasts by reactive derivatives of aromatic amide derivatives. Chem Biol. Interactions, 29, 43-56 (1980). A.E. Aust, K.J. Falahee, J.J. McCormick, and V.M. Maher. Human cell-mediated benzo(a)pyrene cytotoxicity and mutagenicity in human fibroblasts. Cancer Res., 40 4070-4075 (1980).

149. CARCINOGEN DAMAGE AND REPAIR OF DNA IN HUMAN CHROMATIN

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The broad objective of this study is to understand the role of chromatin structure in the distribution of carcinogen-induced damage to DNA and its subsequent repair in human cells. In summary of the present report, DNA repair synthesis within different structural regions of chromatin was measured by a mathematical analysis of the kinetics of digestion with staphylococcal nuclease and DNase I. In addition gel electrophoresis was used to analyze DNA fragments resistant to these enzymes. It was found that UV-induced DNA repair synthesis in cultured human fibroblasts occurs almost entirely in the linker regions of nucleosomes. Following repair synthesis, 80-85% of the repair-incorporated nucleotides are involved in nucleosome rearrangement yielding a more uniform distribution of repair synthesis in linker and core DNA. A small fraction of the repair-incorporated nucleotides (15-20%) are not involved with this rapid rearrangement and remain nuclease sensitive. Neither the distribution of repair synthesis nor the rate and extent of rearrangement were affected by different UV doses (1.5-50 J/m2), the presence of hydroxyurea, or the rate of repair synthesis. Similar results were obtained with two different classes of xeroderma pigmentosum (XP) cells which have been shown to be repair deficient.

150. CHEMICAL CARCINOGEN-INDUCED DNA REPAIR IN HUMAN CELLS

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The overall objective of this project is to understand more about how human cells repair DNA damage induced by carcinogens. During the past year we have focused on two major sets of specific aims. The first involves additional studies on the role of chromatin structure in repair, while the second relates to identifying individual enzymes and other proteins which are involved in DNA excision repair in eukaryotic cells. Work on the role of chromatin structure in excision repair has centered around the phenomenon of rearrangement, i.e., the finding that initially nucleotides incorporated during repair synthesis are staphylococcal nuclease sensitive, and during a subsequent chase period, they become nuclease resistant (appear in nucleosome core particles). With some agents such as 7-bromomethylbenzanthracene and ultraviolet radiation, damage is relatively uniformly distributed between core DNA and linker DNA; with 7-BMBA, rates of removal are similar in core and linker regions. Two possible models might explain these data: one is an unfolding-refolding model of rearrangement in which there are changes in chromatin conformation during DNA excision repair; these changes render the regions undergoing repair temporarily nuclease sensitive, and subsequently they become resistant. A second model suggests that the repair-incorporated nucleotides become nuclease resistant because of sliding. We have tried several approaches to isolate and purify factors involved in repair. One set of experiments involves utilizing a permeable cell system developed by us (Roberts and Lieberman, Biochemistry 18:4499, 1979) to complement permeable xeroderma pigmentosum cells. We have recently isolated a carcinogen-DNA binding protein: the protein recognizes and binds to DNA modified by N-acetoxy-2-acetyl-aminofluorene (NA-AAF), methylnitrosourea, and methylmethanesulfonate, but shows little binding to normal DNA (Moranelli and Lieberman, PNAS 77:3201, 1980). At present the function of this protein is unknown, but we are actively working towards its further purification and characterization.

BIBLIOGRAPHIC REFERENCES: Smerdon, M.J., and Lieberman, M.W. Comparison of the distributions within chromatin of DNA repair synthesis occurring at different times after UV radiation. Biochemistry 19:2992-3000, 1980. Moranelli, F., and Lieberman, M.W. Recognition of chemical carcinogen-modified DNA by a DNA-binding protein. Proc. Natl. Acad. Sci. USA 77: 3201-3205, 1980.

151. INDUCTION OF DNA REPAIR BY CHEMICAL CARCINOGENS

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This is part of a broader project. A summary of this subproject is not available.

152. DNA SYNTHESIS INHIBITION TEST FOR SCREENING OF MUTAGENIC CARCINOGENS

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A test to detect mutagenic carcinogens, based on prolonged inhibition of human cell DNA synthesis by DNA damaging agents, has been proposed. Validation of this test is still to be accomplished. Known carcinogens and structural analogues that are not carcinogens will be evaluated. This will be quantified and its various components will be standardized. The effluents from coal production andutilization facilities will be evaluated by the test for their content of mutagenic carcinogens.

153. MUTAGENICITY TESTS IN BACTERIAL AND MAMMALIAN CELL SYSTEMS AS SHORT-TERM TESTS FOR CARCINOGENESIS (ABBREV)

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OBJECTIVE: Identify possible carcinogenic compounds through short-term tests and obtain information on the interaction of these compounds with cellular DNA by comparing mutagenic and killing responses of bacterial strains deficient in known DNA repair functions.

APPROACH: The following short-term tests are used: a) Salmonella/ mammalian microsome mutagenicity test, b) other back mutagenicity tests in Escherichia coli (strains WP2, AB1157, etc.), c) forward mutagenicity tests in Escherichia coli (streptomycin resistance, nalidixic acid resistance, valine resistance, etc.), d) 6-thioguanine resistance in V-79 cells (Chinese hamster cells cultured in vitro).

PROGRESS: The mutagenic activity of different linear and angular furocoumarins (psoralen and angelicin derivatives) has been identified and compared in bacterial and mammalian cell systems. It has been shown that methyl and other substituent groups in different positions of the molecule may sharply modify the mutagenic activity of angular furocoumarins. Researches are also in progress on the importance in Escherichia coli of polA, polC, recBC chromosomal alleles and of different bacterial plasmids, for regulating the level of mutants induced by UV light and chemical mutagens.

154. RELATIONSHIP OF DNA REPAIR PROCESSES TO MUTAGENESIS AND CARCINOGENESIS IN MAMMALIAN CELLS

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The objective of this research is to determine the role of DNA repair in mutagenesis and carcinogenesis. We aim to vary repair capabilities by (1) using wild-type and repair deficient cell lines; (2) imposing various recovery periods following mutagenic treatment; (3) exposing cells to inhibitors of DNA repair following mutagenic treatment; and (4) varying the mutagenic treatment to cause DNA lesions repairable by different pathways. We have conducted reconstruction experiments to investigate conditions for measuring transformation frequency using colony formation in soft agarose as the end point and to determine the influence of cell density on the expression of mutations to drug resistance. To conduct the reconstruction experiments we have used transformed lines of C3H 10T 1/2 cells, as well as ouabain resistant to the drug with regard to survival and the activity of the Na/sup plus/-K/sup plus/-ATPase, as measured by the incorporation of rubidium. When confluent cells were allowed a four-hour recovery period following mutagenesis, both survival and the frequencies of transformation and mutation increased. However, if logarithmically growing cells were incubated in 0.1% serum for six hours following mutagenesis, no change in either the transformation or mutation frequency was observed as compared to cells incubated in 10% serum. Further experiments are in progress to confirm these results.

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155. STUDIES ON THE MOLECULAR MECHANISMS OF REPLICATION, REPAIR AND MUTAGENESIS OF DNA IN HUMAN CELLS

Riva, S. C., Falaschi, A., Ciarrocchi, G., Pedrini, A. M., Mastromei, G., Consiglio Nazionale delle Ricerche, Laboratorio di Genetica Biochimica ed Evoluzionistica, *Via S. Epifanio 14, 27100 Pavia, Italy*

The summary for this project is under entry no. 63.

156. CYTOTOXIC, MUTAGENIC AND CARCINOGENIC EFFECTS OF ENERGY RELATED AGENTS IN DIPLOID HUMAN CELLS WHICH DIFFER IN DNA REPAIR CAPACITY

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The summary for this project is under entry no. 65.

157. ROLE OF MUTAGENESIS IN CHEMICAL CARCINOGENESIS

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There is increasing evidence to support the hypothesis that somatic cell mutations are causally related to chemical and physical carcinogenesis and that cellular DNA repair processes recognize and repair lesions in DNA caused by the attack of carcinogens, e.g., the frequency of mutations induced in cells derived from xeroderma pigmentosum (XP) patients (who are prone to sunlight-induced DNA damage) is significantly higher than in normal human cells. Although studies have been done to detect, characterize, and quantitate DNA repair following carcinogen attack, few have directly examined the effect of DNA repair processes on the induction of mutations and correlated this with the number and types of DNA adducts formed.

The fundamental purpose of this research is to examine the effect of DNA repair on the induction of mutations by selected carcinogenic agents in normal human cells and in cells derived from persons with genetic predisposition to cancer (classical and variant XP's; ataxia telangiectasia; Fanconi's anemia; etc.). We will: (1) investigate the effect of DNA excision repair processes acting on chemical agents ireduce 'UV-like' repair, 'gamma-ray-like' repair, cross-links, etc; (2) determine the effect of DNA post-replication repair of such damage; and (3) determine, using radioactively-labeled carcinogens and sensitive biochemical techniques, the number and kinds of DNA-carcinogen adducts that correlate with the induction of mutations in these cells. As a corollary, we will: (4) compare the effects of 'simulated sunlight' with those found for 254nm and (5) compare the effects of carcinogens in human epithelial cells those observed in fibroblasts.

A basic understanding of the effect of cellular repair processes on the mutagenic action of carcinogens in human cells is essential for understanding their mechanisms of action.

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158. STUDY OF MODE OF ACTION OF CHEMICAL MUTAGENS

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We have proposed three lines of work to help understand the molecular mechanisms of induced mutation in mammalian cells: 1) Determination of the role of base sequence and of combination with protein in the reactivity of DNA with mutagenic agents; 2) determination, by both in vivo and in vitro studies, of the biochemical mechanisms for the excision repair of chemically induced damage; and 3) study of of the mechanisms by which mammalian cells can replicate past lesions in their DNA. In the coming year we propose to approach these objectives by the following series of experiments: A) Use of phi X174 DNA damaged by reaction with acetoxyacetylaminofluorene and other electrophilic reagents to determine how interaction with different DNA polymerases and metal ions determines the extract nucleotide site at which DNA synthesis is blocked. We

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want to examine the role of the 3'-5 exonuclease in establishing the site of inhibition of synthesis. B) Purification of the apurinic/apyrimidinic nuclease with associated 5'-3' exonuclease activity from lymphoma cells to determine whether both activities are on a single protein molecule and for use of the enzyme(s) as a reagent for the study of mutagen-DNA interactions. We plan to study the behavior of the multi-enzyme systems resulting from combination of this nuclease with bacterial and mammalian DNA polymerase. C) In vivo studies using excision defective xeroderma pigmentosum lymphoblastoid lines to understand the biosynthetic bypass of lesion in DNA by measurements of the amount of single strandedness in DNA isolated from benzoylated naphthoylated DEAE cellulose columns.

BIBLIOGRAPHIC REFERENCES: Strauss, B., Altamirano, M., Bose, K., Sklar, R., and Tatsumi, K. Carcinogen-induced damage to DNA, in (Griffin, A.C., and Shaw, C., eds.) Carcinogens: Identification and Mechanisms of Action. Raven Press, NY 1979, pp. 229-250. Higgins, N.P., and Strauss, B.B. 1979. Differences in the ability of human lymphoblastoid lines to exclude bromodeoxyuridine and in their sensitivity to methyl methanesulfonate and to incorporated (3H)thymidine. Cancer Res. 39:312-320.

159. DNA DAMAGE AND REPAIR AND OTHER SHORT-TERM EFFECTS OF MUTAGENS AND CARCINOGENS (ABBREV)

Parodi, S., Taningher, M., Balbi, C., Bolognesi, C., Cesarone, C., Chiabrera, A., Fassina, G., Pala, M., Russo, P., Vecchio, D., Zunino, A., Istituto Scientifico per lo Studio e la Cura dei Tumori, Division of Chemical Carcinogenesis, *Viale Benedetto XV,10, 16132 Genoa, Italy*

OBJECTIVE: We want to study the short-term effects of mutagens and carcinogens both at DNA and cell morphology levels. The final goal is to improve our understanding of chemical carcinogenesis, or the relationship between initial and late events. This goal also has practical implications in terms of short-term prescreening tests; some of which are followed actively.

APPROACH: In the field of DNA damage and repair the potentialities of two different methods are actively explored: i) 'in vivo' DNA alkaline elution assay, and 2) DNA disentanglement times and viscosimetry with a new model of a toroidal, low shearing, viscometer. The induction of sister chromatid exchanges (SCE) both 'in vitro' and 'in vivo' is also investigated especially under the same experimental conditions that are explored for DNA damage.

The effects of mutagens and carcinogens are also explored at a different level: we work for the possible detection of 'morphological mutant' colonies, with techniques of scanning microscopy plus objective densitometric and geometric analysis. This work is pursued in close collaboration with the Institute of Electric Engineering (Division of Bioengineering) of the University of Genoa.

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160. CELLULAR DNA REPAIR MECHANISMS AND MUTAGENESIS

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The proposed research will investigate the methods of maintenance of the integrity of the chromosome. There are a number of environmental factors which may alter the functions of a cell, either by interfering with a specific pathway of metabolism or by altering or damaging the cell's content of information, the genome, producing mutagenesis. Among these are short wavelength irradiation and chemical agents which alter the structure of DNA. The purpose of this research will be to investigate protective mechanisms of biology of the cell used in response to such insult. The role of cell repair functions in mutagenesis and carcinogenesis will also be studied. Experiments will be done in an in vitro bacterial system capable of repair synthesis of DNA to study mechanisms responsible for the preservation of the message. Repair synthesis in toluene-treated E. coli is available to small molecules as well as to macromolecules. This affords control over many enzymatic reactions occurring during repair. This system also offers the advantage of isolation of the intermediates and products of repair synthesis under conditions of regulated metabolism. Efforts will be directed towards extending the work to include development of an open or lysate system that will afford greater definition and macromolecular access. A direct evaluation of repair steps in repair-deficient procaryotes and eucaryotes will be done. The mechanism of action of drugs such as the antitumor agent, bleomycin, can be investigated to advantage in vitro. Mutants defective in repair processes can be used to define the required steps of repair in response to this agent. This agent will also be tested for mutagenicity. It will be a prototypic agent for evaluating the relationship of repair and carcinogenesis in tissue culture. The combination of genetic and molecular techniques should allow us to correlate processes occurring in vitro to those in vivo and define a common series of events that are of importance for maintenance of the chromosome in procaryotes and more complex organisms.

BIBLIOGRAPHIC REFERENCES: Moses, Robb, E. and Beaudet, Arthur L., Apurinic DNA Endonuclease Activities in Repair-Deficient Human Cell Lines, Nucleic Acids Research, 5:463-473 (1978).

161. MUTAGENESIS AND REPAIR OF DNA

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The summary for this project is under entry no. 64.

162. A STUDY OF MUTAGENESIS BY CHEMICAL CARCINOGENS

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This proposal is concerned with mutagenesis and carcinogenesis induced by aromatic polycyclic hydrocarbons which are environmental pollutants. The experiments described will show whether, and how, DNA adducts formed from the proximate carcinogens produce mutations. Our study offers a new approach: we will use the mammalian virus SV40, with its DNA modified in vitro by treatment with the proximate hydrocarbon carcinogen. The modified and characterized SV40 DNA vector will then be studied in infected cells from which we will learn the molecular nature (base change, insertion, deletion) and location of mutations caused by the hydrocarbon adducts. Our studies will also help to elucidate the role of DNA repair in mutagenesis. We will study both pre- and post-replicative repair and will determine whether the repair process itself is error-prone. A close correlation between mutagenesis and carcinogenesis will be made possible by examining the extent of mutagenesis of SV40 in the same cells in which transformation by the hydrocarbons will be studied. This last approach will also provide a basis for the screening of potential carcinogens.

163. ENVIRONMENTAL MUTAGENESIS AND DNA REPAIR

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Mutagen-sensitive (mus) mutants of Drosophila melanogaster are being isolated and characterized for potential alterations in spontaneous and induced mutation frequencies and defects in DNA repair ability. Current studies employ selected monofunctional alkylating agents, which vary with respect to Swain-Scott constants, and include methyl methanesulfonate, ethyl methanesulfonate, methyl nitrosourea and ethyl nitrosourea.

The sex-linked recessive lethal test and the X-linked white eye color locus are being employed to monitor the effect of specific mus loci on alkylation-induced mutation production. Current experiments are focused on the effects of the excision-defective mei-9 and mus(2)201 loci.

In vitro cell culture systems prepared from embryos of wild type and mus strains are being employed to study both normal DNA synthetic capacity and DNA repair capability

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following damage by the alkylating agents. Repair studies utilize both autoradiographic determinations of unscheduled DNA synthesis (UDS) and alkaline and neutral sucrose gradient centrifugation to identify specific defects in the repair of alkylation-induced lesions.

BIBLIOGRAPHIC REFERENCES: P.D. Smith, R.D. Snyder and R.L. Dusenbery. 1980. Isolation and Characterization of repair-deficient mutants of Drosophila melanogaster. In DNA Repair and Mutagenesis Eukaryotes (DeSerres, Generoso and Shelby, eds.) pp. 175-188.

164. MUTAGENESIS AND CARCINOGENESIS

Chambers, R. W., Bhanot, O. S., Zawadzka, H., New York University, School of Medicine, Dept. of Biochemistry, *100 Washington Sq. E., New York, New York*, 10003, United States of America

The long range goal of this research is to prepare site-specific covalent adducts that represent the products formed when various carcinogens react with DNA in vivo and to examine the mutagenic effect of these lesions both in vivo and in vitro in order to define the precise series of biochemical events that leads to mutations. Ultimately we would like to know whether this series of events is prerequisite to tumor production. Our initial objective is to answer the following specific questions: (1) Which of the covalent adducts produced in DNA by reactions with different carcinogens are mutagenic? (2) What type of mutation (base substitution, frameshift, large deletion or rearrangement) does each kind of DNA adduct produce? (3) What roles do various DNA repair systems play in producing these mutations? (4) Is there any correlation between a particular type of DNA adduct produced by known carcinogens and the carcinogenic index of that carcinogen.

We have developed a system using bacteriophage phiX174 that we believe is capable of answering these questions. We are introducing different potentially mutagenic lesions into single preselected sites in gene G (an essential gene) by a combination of chemical and enzymatic syntheses. The expression of these site-specific modifications is being examined in vivo by transfection of spheroplasts derived from various E. Coli mutants that are defective in certain DNA repair systems. Mutant virus present in the spheroplast lystac is detected and propagated using a phiX-sensitive host carrying a functional copy of phiX gene G on a plasmid. This phiXG system is permissive for all types of mutation in gene G. Viral DNA carrying mutations is isolated from the rescued phage and sequenced in the region of interest to define the precise nature of the mutations that have occurred in response to the original site-specific covalent modification.

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165. INDUCTION AND REPAIR OF DNA DAMAGE AFTER IRRADIATION OR TREATMENT WITH ANTI-CANCER DRUGS

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The summary for this project is under entry no. 99.

166. AN IMMUNOLOGICAL APPROACH TO STUDY DNA DAMAGE AND REPAIR BY IRRADIATION AND CHEMICAL CARCINOGENS

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The summary for this project is under entry no. 56.

167. RADIATION AND CHEMICAL IN VITRO MALIGNANT TRANSFORMATION

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The summary for this project is under entry no. 77.

168. REPAIR OF DNA TREATED WITH GAMMA-IRRADIATION AND CHEMICAL CARCINOGENS

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The summary for this project is under entry no. 57.

169. INTERACTION OF ULTRAVIOLET LIGHT AND CHEMICAL CARCINOGENESIS

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The summary for this project is under entry no. 52.

170. RADIATION AND CHEMICAL DAMAGE

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The summary for this project is under entry no. 71.

171. PRIMARY BIOCHEMICAL ALTERATIONS LEADING TO NEOPLASTIC CONVERSION

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This program is designed to identify primary chemical and structural alterations in DNA leading to neoplastic transformational in human cells and rodent cells and quantitate the repair response of the cells to agents which induce the primary alterations. The immediate objectives are to develop methods to transform human epithelial cells and to compare the type of lesions caused by low level ('nontoxic') insults to DNA with high level (toxic) insults and measure the cell repair response to these lesions. The lesions currently of most interest are DNA-protein crosslinks including both their induction and repair.

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172. TOXICITY IN DNA REPAIR DEFICIENT AND PROFICIENT CELLS

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The aim of this project is to investigate the roles of DNA repair and DNA replication in predicating toxicity (cell killing and neoplastic transformation) in repair-deficient and repair-proficient 10T1/2 cells. We have found that C3H 10T1/2 cells can be made DNA repair-deficient by depriving them of isoleucine. Compared to repair-proficient 10T1/2 cells (isoleucine-sufficient), repair-deficient cells are more sensitive to the cell killing and transforming effects of directly acting alkylating agents such as N-methyl-N'-nitrosoguanidine. In this project we will determine whether the DNA repair deficiency involves excision or replication repair (or both) and, by utilizing synchronized cells, we will examine the role of DNA replication in causing toxicity. The competence of DNA excision repair will be evaluated in G1 phase cells by measuring single strand breakage and gap-formation, removal of alkylated bases, repair DNA synthesis, and ligation. Post-replication repair will be evaluated in S phase cells by determining the rate of formation and size of primary pieces and by measuring the accumulation of gaps in DNA. Toxicity is assessed by determining the colony-forming efficiency of treated and control cells.

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173. THE ROLE OF DNA REPAIR MECHANISMS IN THE ETIOLOGY OF CANCER

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The study of defective repair of MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) produced damage was extended. One fifth (14/70) of the human tumor cell strains tested comprises a group of cell strains (Mer minus) that differs from the remaining 56 tumor strains (Mer plus) or from normal human fibroblasts (also Mer plus) in the following ways: 1) Inability to excise O to the 6th power-methylguanine from their DNA after being treated with MNNG; 2) relative inability to support the growth of MNNG damaged adenovirus 5; and 3) sensitivity to being inactivated by MNNG or BCNU (Bis-chloroethyl-nitrosurea) in a reproductive way. The results may be relevant to uncovering the molecular reasons for success of certain bifunctional alkylating chemotherapeutic agents (BCNU, CCNU).

BIBLIOGRAPHIC REFERENCES: Day, Rufus S., III, and Chuck H.J. Ziolkowski: Four human brain tumor cell strains have deficient host-cell reactivation of N-methyl-N'-nitro-N-nitrosoguanidine damaged adenovirus 5. Nature 279: 797-799, 1979. Day, Rufus S., III, Ziolkowski, C.H.Z., Scudiero, D.A., Meyer, S.A., and Mattern, M.R.: Human tumor cell strains defective in the repair of alkylation damage. Carcinogenesis 1: 21-32, 1980.

174. STUDY OF DNA REPAIR SYNTHESIS, WITH SPECIAL REFERENCE TO DNA REPAIR LEVELS, AGING AND SPECIES DIFFERENCES

Takayama, S., Ishikawa, T., Ide, F., Cancer Inst. Hospital, Dept. of Experimental Pathology, *1-37-1 Kamiikebukuro, Toshima-ku, Tokyo 170, Tokyo, Japan*

Three methods were devised for measuring DNA repair synthesis at a cellular level in vivo or in short-term organ cultures. In these systems unscheduled DNA synthesis could be demonstrated quantitatively by grain counts over the nuclei of cells treated with various carcinogens. Comparative studies were made on DNA repair levels in relation to aging and species differences.

For studies on aging, part of the skulls of small fish (Oryzias latipes) of various ages was removed and the brains of the living fish were treated with 4-nitroquinoline-1-oxide (4NQO) and (methyl-3H) thymidine (TdR). Freshly removed retinas and tracheas of rats and golden hamsters of various ages were treated with 4NQO and (methyl 3H) TdR in vitro. The levels of unscheduled DNA synthesis were studied autoradiographically. These studies showed that there was no age-associated change in the DNA repair levels of the ganglion cells of the central nervous systems or the tracheal epithelial cells.

For studies on species differences in DNA repair levels, pieces of retina and trachea from six species (mice, rats, golden hamsters, guinea pigs, pigs, and cows; all young adult males) were treated with three carcinogens in short-term organ cultures. The results showed that different species differed considerably in their levels of unscheduled DNA synthesis. Moreover, the relative efficiencies of DNA repair in the six species depended on the carcinogen tested. For example, mouse cells, which were previously considered to show very low levels of excision repair in cell culture, were found to show active unscheduled DNA synthesis in short-term organ cultures of retinas and tracheas.

175. IDENTIFY HETEROZYGOUS CARRIERS OF DNA REPAIR DEFECTS

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Develop a sensitive radioimmunoassay for determination of the loss of adducts. Raise antibodies against chemical carcinogens complexed human DNA complexed with serum albumin in rabbits. Consider the use of monoclonal antibody production as a preferred alternative. Standardize mitogen activation methods to permit the use of activated lymphocytes and demonstrate that lymphoblastoid cultures reflect the repair capacity of the donors. Further develop the existing BND cellulose test to make it applicable for chemical use.

176. DNA REPAIR MECHANISMS IN HUMAN CELLS

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Our broad objective is to elucidate the role of DNA repair in carcinogenesis. Our research focuses upon biochemical mechanisms and genetic control of excision-repair (ER) in mammalian cells. This is important for understanding hereditary diseases like xeroderma pigmentosum (XP) in which defective ER is correlated with a high incidence of cancer.

We are improving the accuracy of ER patch size measurements applying the bromouracil density shift method to DNA fragments of defined length (e.g. a 172 bp sequence from monkey alpha DNA). Results will be compared to those obtained with the bromouracil photolysis method to resolve apparent discrepancies and to test the hypothesis that patch size depends upon the type of lesion being repaired. Having found that the antileukemic drug, cytosine arabinoside, inhibits rapair synthesis in UV irradiated cells by decreasing the number, but not the size, of patches we plan to investigate the mechanism by which this drug interferes with patch ligation.

We will measure the rate and extent of repair synthesis and lesion removal in human cells containing quantifiable lesions (including ultraviolet induced pyrimidine dimers, angelicin monoadducts, psoralen monoadducts and interstrand crosslinks, and estragol adducts) to assess the overlap of ER pathways, differences in affinity of ER enzymes for different lesions, and effects that one type of lesion may have upon repair of another.

The influence of chromatin structure on ER will be analyzed by comparing ER of lesions randomly located in DNA with that of lesions restricted to 'linker' regions. The chromatin form of monkey alpha DNA and Tetrahymena rDNA will be used to examine the effects of lesions on nucleosome stability and the accessibility to lesions to repair enzymes such as T4 endonuclease V. XP cells will be tested for impaired accessibility to ER of lesions in large domains of chromatin.

The cloned denV gene of bacteriophage T4 (coding for endonuclease V) will be inserted into suitable vectors to study its expression in human XP cells. We will also attempt to complement ER defects in XP cells by DNA mediated transfer of genes from normal human cells.

177. DNA REPAIR IN SLIME MOLD

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The summary for this project is under entry no. 92.

178. GENETIC DISSECTION OF DNA REPAIR

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The human skin disease Xeroderma pigmentosum (XP) demonstrates direct linkage between defective repair of damaged DNA, induction of mutations, and induction of cancers. An elucidation of the basis for the genetic heterogeneity of XP is necessary if the functional relationships between DNA repair, mutagenesis, and carcinogenesis are to be fully understood. Therefore, the specific objectives of this project will be to: (1) determine the genetic basis of the heterogeneity in XP complementation groups; (2) determine the number of different genes which may be involved in the expression of the Xeroderma pigmentosum phenotype; (3) determine the chromosomal location of the gene(s) responsible for XP; and (4) determine the specific genetic contribution made by mouse cells to the XP cells which results in genetic complementation of their DNA repair capability. Our technical capacities to pursue this study reside in the ability to generate and genetically analyze man x mouse somatic cell hybrids for the presence of each human and mouse chromosome and the ability to repair DNA damage. The assays for DNA repair include: (1) bromodeoxyuridine photolysis; (2) radiochromatography; and (3) molecular weight analysis. The use of somatic cell hybrids will make it possible to determine gene-chromosome assignments and to dissect this complex polygenic system by isolating its component parts.

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179. CARCINOGEN-CELL GENOME INTERACTION

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The principle aims of the research of this laboratory are to assess the effects of energy-related pollutants (chemicals and radiation) on cell gene components which are related to cancer formation and to investigate the genetic defense mechanisms of the cell against environmentally hazardous agents. Experimental approaches currently undertaken for the tasks follow. Effects of gene perturbation by chemicals and radiation are studied with the use of the DNA transfection method which involves insertion of an exogenous DNA molecule carrying oncogenic information and expression of this DNA in the cell. We have devised highly specific and sensitive techniques for studying the transposable gene elements in mammalian systems which are currently considered to be an important aspect of cancer research. Enzyme systems associated with replication and repair of DNA and also with chromatin functions are studied in aging mammals that show a high incidence of cancer, presumably due to the cumulative effect of environmental insults on the cell genome. We have found in the spleen and brain of old rats a decreased quantity of DNA polymerase activity which apparently led to increased numbers of lesions in the DNA. Through an interagency agreement, the National Cancer Institute has supported this laboratory in two other specific studies: (1) restriction of leukemia viruses by host Fv-1 gene, and (2) primer-tRNA-binding RNA molecules of human cancer cells. These four projects are independent but beneficial to one another in research progress.

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180. ONCOLOGICAL STUDIES - DNA REPAIR

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Further experimentation was conducted in order to study the effects of covalently bound adducts of the carcinogen N-hydroxy-l naphthalamine (N-OH-lNA) in producing, or leading to precursors of, DNA strand scissions. Calf thymus DNA reacted at pH 5.0 with tritiated N-OH-lNA was previously shown to contain adducts which were expressed as DNA single strands breaks in alkali (0.1N NaOH), as measured by velocity sedimentation in a Beckman Model E analytical ultracentrifuge equipped with UV optics and a photoelectric scanner. We calculated a value of 0.075 DNA single strand scissions in alkali per N-OH-lNA adduct.

Other data suggested that some of the DNA alkylation products might lead to depurination and thus to DNA strands containing apurinic sites. To investigate this possibility, we treated samples of calf thymus DNA containing N-OH-lNA adducts with AP endonuclease (provided by Dr. C. Springgate, Tulane Medical School), which is specific for apurinic-apyrimidinic sites in native DNA. Endonuclease treated DNA samples were then dialyzed against 95 percent formamide in 0.015 M EDTA, pH 7.0, incubated in the formamide solution at 37 degrees C for 2 hours in order to denature the DNA, dialyzed exhaustively to remove formamide, and then analyzed by velocity sedimentation using the analytical ultracentrifuge. Samples of purified lambda phage DNA, depurinated by heating at 70 degrees C for various times at pH 5.0, were used to evaluate the endonuclease and formamide denaturation treatments. Preliminary data with N-OH-lNA treated calf thymus DNA suggest that only a small fraction of N-OH-lNA adducts leads to loss of a base under these conditions.

D. OTHER STUDIES OF DNA REPAIR

181. TESTING DNA DAMAGE AND REPAIR WITH SOMATIC CELL HYBRIDS

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This project focuses on some hitherto unexplored potentials of somatic cell hybridization to evaluate in strict genetic terms the biological effect of radiations and radiomimetic substances on the human diploid cells freshly derived from the propositi to be tested. The rationale of this methodology is essentially the same as that employed by Goss and Harris for the mapping of human genes with the so-called radiation co-transfer test. However, in order to make the test amenable to practical usage, we have restricted the screening for radiation damage to the small target site of the human X-chromosome long arm between Xq26 and Xqter which includes the loci for HGPRT and G6PD. White blood cells from normal individuals or from patients affected by or highly susceptible to various malignancies are submitted to a standard set of mutagenic treatments and then fused - by means of polyethylene glycol or Sendai virus - with rodent cells deficient in both HGPRT and G6PD. Preliminary studies have indicated that under these circumstances, actively growing hybrid cells isolated in the HAT selective medium within two weeks from the fusion experiment, have all regularly retained the human HGPRT while the retention of G6PD depends upon the co-transference of the unbroken target site into the hybrid genome. Thus the relative frequency of G6PD positive hybrid cells provides a quantitative measure of the radiation effect at the level of functional chromosomal DNA. Variations of this methodology will be applied to make it suitable also to the measurement of DNA repair. Depending upon the type of protocol and of the human material chosen, this new experimental procedure is expected to yield data on the individual variation in the susceptibility to DNA damage/repair and to offer an altogether new handle for the screening of hidden genetic damage in individuals chronically exposed (for whatever reason) to mutagenic carcinogenic agents.

182. GENETIC ANALYSIS OF DNA REPAIR IN MAN WITH CELL HYBRIDS

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OBJECTIVES: The long range goal of this project is to investigate the expression and individual genetic components involved in DNA repair in man. The primary objectives of this project are: 1. To genetically dissect the DNA repair systems in man. 2. To identify the number and kinds of genes required for DNA repair. 3. To assign these genes to specific human chromosomes. 4. To identify and determine the chromosomal assignments(s) of the genetic defect in xeroderma pigmentosum (XP).

MAJOR FINDINGS: Man x mouse somatic cell hybrid lines were generated between human cells derived from different individuals with normal DNA repair capacity and established mouse cell lines. Primary hybrid clones isolated under the conditions employed preferentially segregated human chromosomes and retained the full complement of mouse chromosomes. Since the magnitude of DNA repair in the mouse cells is less than 10% of the excision repair seen in human cells, the human DNA repair components are distinguishable from the mouse DNA repair components in the man x mouse somatic cell hybrids.

The hybrids assayed for excision repair ability appear to group themselves into one of three categories: 1. Those having a magnitude of repair similar to human cells. 2. Those having mouse-like repair. 3. Hybrids intermediate between the two.

Segregation of the ability to repair ultraviolet (UV) damage in 41 primary hybrid clones has been tested for linkage with 30 enzyme markers representing genes known to be assigned to each of the 21 different human chromosomes. Concordant segregation was not observed between DNA repair and those other enzyme markers indicating that the assignment of a gene(s) required for the ability to repair UV-induced DNA damage can be excluded from all but three human chromo-

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somes. Cytogenetic analysis of informative clones will indicate whether the gene(s) required for repair of UV-induced DNA damage are carried by 1, 2 or 3 chromosomes.

An important question that must be answered in order to interpret the data is whether the mouse input cells can contribute anything to the excision repair seen in the hybrid cells. This question was pursued by fusing mouse cells to human cells defective in DNA repair derived from XP patients. (Text Abridged.)

183. DNA REPAIR IN NORMAL AND REPAIR DEFICIENT HUMAN CELL LINES

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It is anticipated that research efforts will be expended in two general areas: 1) Purification and Characterization of a Pyrimidine Dimer DNA Glycosylase from the nuclear membranes of normal human fibroblasts. Antibodies to this enzyme will be used to determine its levels, distribution and nature. 2) The cloned Escherichia coli genes uvrA, uvrB and uvrC will be integrated into composite vectors consisting of SV40 DNA and pBR322 components to attempt genetic complementation of xeroderma pigmentosum homozygous cell lines by transfection. Reversion of these repair deficient cell lines to repair proficiency will be selected by growth in a highly selective medium consisting of ethylmethanesulfonate and anthramycin.

184. DNA REPLICATION AND DNA REPAIR IN MYCOPLASMATALES

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DNA replication and DNA repair in Acholeplasma laidlawii would be studied with the following specific aims: The initiation process of DNA replication would be studied using amino acid starvation and antibiotic inhibition of reinitiation. Isolation of restriction fragments containing the origin would be done using restriction fragment analysis of cells radioactively labeled at the origin. Such fragments would be cloned in E. coli K12 strains, thereby creating origin plasmids for detailed structural studies of origin DNA and for in vitro studies of initiation. Studies of the elongation process would center on isolation and characterization of Okazaki fragments, on the rate of chain elongation, and on autoradiographic evidence for bidirectional vs. unidirectional replication. The properties of A. laidlawii folded chromosomes as related to the DNA replication cycle would be analyzed via sedimentation and electron microscopic techniques. A. laidlawii plasmids would be characterized, and enzymes related to DNA structure and function would be sought and isolated. Genes for such enzymes would be isolated via cloning of A. laidlawii DNA restriction fragments in E. coli K12 strains auxotrophic for DNA functions.

BIBLIOGRAPHIC REFERENCES: The Escherichia coli dnaB protein is required for initiation of chromosomal DNA replication. 1979. F.K. Fujimura, J.W. Zyskind, and D.W. Smith. Cold Spring Harbor Symp. Quant. Biol. 43:559. Isolation and mapping of plasmids containing the Salmonella typhimurium origin of DNA replication. 1979. Judith W. Zyskind, L. Thomas Deen, and Douglas W. Smith. Proc. Natl. Acad. Sci. USA 76:3097.

185. IDENTIFICATION OF HETEROZYGOUS CARRIERS OF DNA REPAIR DEFECTS

Chaganti, R. S., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

OBJECTIVE: Develop, using chromosome instability (chromosome breakage and sister chromatid exchange) as the end-point, methods for the detection of the heterozygous genotypes for the genes causing the autosomal recessive disorders such as Fanconi's anemia. In addition, other syndromes such as AT, XP, may be tested as a correlate; however, the primary focus will be on determining a methodology which will detect heterozygote genotypes of FA.

186. DNA REPAIR AND CONTROL OF DNA REPLICATION AND SYNTHESIS AND DEGRADATION OF POLY (ADP-RIBOSE)

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DNA Repair and Control of DNA replication: Objective: (1) Molecular mechanisms of DNA repair in T4 phage. (2) Mechanism of shutoff of host DNA replication in the E. coli-Tr phage system.

APPROACH: Develop in vitro system.

PROGRESS: (1) Genetic components in DNA repair defined. (2) Host rep function inhibited after T4 infection.

Poly (ADP-ribose): Objective: Study of the enzymes involved in Poly(ADP-ribose) metabolism and the biological function of poly(ADP-ribose).

APPROACH: (1) Purify and study the property of the enzymes. (2) Analysis of poly(ADP-ribose).

PROGRESS: Both poly(ADP-ribose) polymerase and glycolydrolase have been purified to homogeneity.

187. ENZYMES AND REACTIONS FOR REPAIR OF DNA IN HUMAN CELLS

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The objective of the proposed research is to identify and characterize the enzymes and reactions involved in excision-repair of DNA in human cells exposed to either radiation or alkylating agents. Efforts will continue to focus on the critical first step in excision, namely recognition of specific lesions by repair endonucleases or N-glycosylases. Previously, I have partially purified from cultured human lymphoblasts (CCRF-CEM line), an endonuclease acting specifically on DNA irradiated by UV or x-ray and an N-glycosylase acting specifically on 3-methyladenine in alkylated DNA. I now plan to purify both these enzymes from human placenta in quantities adequate for extensive characterization, and for use as reagents. Effects will be made to identify the specific target-lesion recognized by the UVX-endonuclease. An immuno-specific assay will be developed for this enzyme, and cell extracts from individuals with 'DNA-repair syndromes' will be screened for enzyme deficiency. Purified enzyme will be used as a specific probe for UVX-endonuclease sensitive sites in DNA from irradiated normal and mutant human fibroblasts to determine if the UVX-endonuclease or its target-lesion is implicated in radiation sensitivity. Similar studies already underway with lymphoblast enzyme will be carried out with purified placental 3-methyladenine-DNA glycosylase to determine if other target-lesions exist or if it is implicated in one of the human 'DNA-repair syndromes.' Both enzymes will be used to probe the kinetics, dose dependence and effects of inhibitors on repair of specific lesions in normal and mutant cells. Knowledge gained through this program will contribute to an understanding of the enzymatic mechanisms for excision repair, and also indicate their involvement in human genetic disorders associated with sensitivity to genotoxic agents and elevated incidences of cancer. Additionally, if resistance of tumors to radiation or radiomimetic drugs involves these excision pathways, this knowledge may well suggest modified approaches to cancer therapy.

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188. STUDY OF ERROR-PRONE DNA REPAIR PATHWAYS IN MAMMALIAL CELLS

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This is part of a broader project. A summary of this subproject is not available.

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189. CONTRIBUTION OF DNA DAMAGE TO CYTOTOXICITY

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No summary has been provided to the Smithsonian Science Information Exchange.

190. MOLECULAR BASIS OF CHROMOSOME ABERRATIONS

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This project studies the molecular mechanisms of chromosomes aberration formation through a multiparameter approach that will allow correlations to be made between specific DNA lesions and specific chromosome aberraton types. We combine the use of DNA repair deficient cell lines, DNA modifying enzymes, alkaline and neutral DNA elution, and premature chromosome condensation to understand how DNA lesions become expressed as chromosome aberrations.

191. NEW SUBSTRATES AND ASSAYS FOR DNA REPAIR AND RECOMBINATION

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The long-range objective is biochemical characterization of one or more DNA repair-recombination pathways, including identification and analysis of the requisite enzymes and intermediate DNA structures, and reconstitution of the systems from purified components. The system uses non-replicating lambda phage DNA as the substrate and Escherichia coli as the source of repair and recombination enzymes. It thus combines molecular simplicity with genetic flexibility, and permits detailed analysis of both in vivo and in vitro events. The immediate goals, and the methods to be used, are as follows: (1) Development of new recombination assays, suitable for biochemical characterization of intermediate steps: a. (restriction enzyme) - (gel electrophoresis pattern); b. transfection infectivity; c. coupled (restriction enzyme) -exonuclease; d. DNA transfer by lac repressor-operator binding. (2) Determination of recombinogenic DNA lesion best suited for pathway characterization (alternatives: a. thymine dimers; b. non-dimer UV (254 nm) photoproduct; c. misincorporated uracil; d. 'hyper-Rec' lesion.) (3) Determination of modes of action of Rec A protein and DNA gyrase. (4) Isolation of mutant strains hyperefficient in repair and/or recombination.

192. POSTREPLICATION AND RECOMBINATION REPAIR OF DNA

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This is part of a broader project. A summary of this subproject is not available.

193. NEUROSPORA DNA REPAIR

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Eukaryote DNA repair systems are important in repair of radiation and chemical damage, in recombination, and probably in cancer induction and in normal DNA and RNA synthesis. The role of DNA polymerase is central to this repair and likely to be different in prokaryotes and eukaryotes. Using biochemical and genetic techniques, the DNA polymerases in the fungus Neurospora crassa are being studied.

Our results indicate that Neurospora has at least two separable DNA polymerase activities. While these activities are separable on hydroxyapatite chromatography and on isoelectric focusing, they are very similar in salt, Mg(III) and pH optima and show identical chromatographic behavior on Sepharose-Blue Dextran, DEAE, and DNA-cellulose. Further characterization of these activities will be undertaken. To understand their biological role we will examine repair-defective mutants, DNA stop mutants and various stages in the life cycle for changes in these DNA polymerase activities.

Characterization of possible DNA repair-defective mutants has shown that 11 new isolates are from at least 7 and probably 11 new genes. Most are sensitive only to UV. Two are sensitive to X rays. Two mutants which disrupt meiosis have also been shown to be sensitive to a broad spectrum of mutagenic agents suggesting that they are repair defective.

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194. DNA REPAIR - HUMAN E. COLI PHOTOREACTIVATING ENZYMES

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Photoractivating enzymes (PREs) play a unique role in DNA repair by their specific, light-dependent monomerization of cyclobutyl pyrimidine dimers in DNA. We shall determine the structure of E. coli PRE, both apoprotein and 'cofactor', and study their relation to the binding and photolytic reactions. We shall isolate large quantities of the mammalian PRE and determine its structure.

The specificity of the enzyme allows its use as a dimer probe: if detrimental biological effects of UV can be reversed in a true photoenzymatic reaction, dimers played a role in the production of that damage. Although mammalian tissues contain PRE, expression of the phr gene in cells in culture has stringent requirements. We have thus developed a method of polyethylene glycol insertion of E. coli enzyme into mammalian cells. This should allow extension of the 'PR test' to cells with low intrinsic PRE levels (e.g. xeroderma cells, rodent cells) or to conditions unfavorable for PRE production.

We are using an alkaline agarose gel technique for measuring dimers in (non-radioactive) human skin. We shall examine repair by excision and photoreactivation in human skin exposed to light from different sources; we shall obtain action spectra for dimer production in leukocytes, and in mouse and human skin.

BIBLIOGRAPHIC REFERENCES: Sutherland, B. M. and S. G. Hausrath (1980). Insertion of E. coli Photoreactivating Enzyme into V79 hamster cells. Nature 286: 510-511. Sutherland, B. M., I. Kochevar and L. Harber (1980). Pyrimidine Dimer Formation and Repair in Human Skin. Cancer Res. 40: 3181-3185.

195. DNA REPAIR IN E. COLI

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This is part of a broader project. A summary of this subproject is not available.

196. DNA MISMATCH REPAIR IN TRANSFORMATION AND MUTAGENESIS

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This project will investigate DNA base mismatch correction in Diplococcus pneumoniae. Such repair occurs after DNA-mediated genetic transformation and after potentially mutagenic base substitution in DNA replication. In transformation, single-site markers are integrated with different efficiencies because a system, called Hex, recognizes mismatches in the intial heteroduplex product of integration and removes the donor portion with a frequency depending on the mismatched base pair formed. Hex(minus) mutants give only high integration efficiency. The molecular mechanism of the Hex system will be determined in vivo by following the biochemical fate of a purified DNA donor fragment containing the amylomaltase gene. This gene has been extensively analysed genetically. By design of a suitable substrate and use of cell extracts lacking nonspecific nucleases, we hope to demonstrate mismatch repair in vitro. The enzymatic mechanism of the repair process could then be analyzed. Of particular importance is how the system recognizes and excludes the donor contribution to the mismatch.

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Strains that lack the mismatch repair system show higher spontaneous mutation rates. The system apparently repairs DNA replication errors by recognizing base mismatches and removing the wrong base in the nascent strand. By analysing reversion rates of mutations in the amylomaltase gene, the mismatch specificities of mutational correction and integration efficiency will be compared. Mismatch repair, as indicated by the occurrence of gene conversions, is widespread among living cells. The proficiency of this system could be a major factor in determining spontaneous mutation rates.

The impact of DNA methylation on spontaneous mutation rates will also be ascertained. If ageing is due to the accumulation of mutations, mismatch repair could be important for longevity and for the prevention of age-related diseases such as cancer and heart disease. An in vitro assay will make the study of mismatch repair accessible also in human cells.

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197. MOLECULAR STUDIES ON THE MECHANISMS OF HUMAN DNA REPLICATION, REPAIR AND MUTAGENESIS

Falaschi, A., Riva, S., Spadari, S., Pedrali Noy, G., Ciarrocchi, G., Pedrini, A. M., Cobianchi, F., Consiglio Nazionale delle Ricerche, Laboratorio di Genetica Biochimica ed Evoluzionistica, *Via S. Epifanio 14, 27100 Pavia, Italy*

OBJECTIVE: The study of the molecules involved in the advancement of the growing fork and in the repair of damage of human DNA should give essential information for the understanding of the modes and causes of uncontrolled cell proliferation.

APPROACH: a) Study of the molecules easing the replication of double-stranded DNA in vitro by purified alpha-polymerase; b) study of the mode of action of a specific inhibitor of DNA replication in animal cells, aphidicolin; c) attempts at establishing an in vitro assay of the repair of UV damage to isolated metaphase chromosomes.

PROGRESS: a) A DNA-dependent ATPase having the properties of a DNA unwinding enzyme and a DNA binding protein have been isolated from mammalian cells; either protein, by different mechanisms, enhances the rate of advancement of DNA polymerase alpha on double-stranded areas by factors of up to 30. When both molecules are positioned in vitro on structures mimicking a growing-fork condition, greater stimulations are attained than the sum of the effect of either alone, indicating some form of interaction. b) Aphidicolin blocks specifically the alpha polymerase by competitive inhibition with dCTP and mixed inhibition with the other triphosphates; its specificity allows a simple measure in vivo of the DNA repair synthesis performed by beta-polymerase; the action of the drug is reversible and allows a good synchronization of the cultures. c) Isolated human metaphase chromosomes are substrates for 'repair type synthesis' in vitro by human cell extracts, but such synthesis does not appear to be enhanced by the previous UV-irradiation.

198. DNA REPAIR, MUTATION AND TRANSFORMATION IN HUMAN LYMPHOID CELLS

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This is part of a broader project. A summary of this subproject is not available.

199. DNA REPAIR AND COLON CANCER

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No summary has been provided to the Smithsonian Science Information Exchange.

200. MECHANISMS OF DNA REPAIR

Duker, N. J., Temple University, School of Medicine, Dept. of Pathology, *3322 Broad St., Philadelphia, Pennsylvania,* 19122, United States of America

This is part of a broader project. A summary of this subproject is not available.

201. HETEROZYGOUS CARRIERS OF DNA REPAIR DEFECTS

Grossman, L., Johns Hopkins University, School of Hygiene & Public Health, Dept. of Biochemistry, *615 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

OBJECTIVE: Scale up the purification of three human placental enzymes to homogeneity suitable for monoclonal antibody production. Particular attention will be paid to the specificity of the antisera.

202. DNA SYNTHESIS AND REPAIR IN VITRO

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This is part of a broader project. A summary of this subproject is not available.

203. DNA REPAIR AND ITS ROLE IN CARCINOGENESIS (MICE, E. COLI)

Belman, S., New York University, School of Medicine, Dept. of Environmental Medicine, *100 Washington Sq. E., New York, New York,* 10003, United States of America

This is part of a broader project. A summary of this subproject is not available.

E. DNA POLYMERASES AND OTHER ENZYMES

204. DNA POLYMERASES OF HERPESVIRUSES AND RNA TUMOR VIRUSES IN HUMAN CANCER CELLS

Allaudeen, H. S., Bertino, J. R., Yale University, School of Medicine, Dept. of Pharmacology, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

With a view to obtain evidence for the viral etiology of human cancer, we have been examining samples from patients with different kinds of cancer for tumor virus markers such as reverse transcriptase of RNA tumor viruses and a DNA polymerase induced by herpesviruses (including Epstein-Barr virus (EBV)). We have examined twenty-four samples thus far; four of the leukemic leukocytes from AML patients had low but detectable levels of reverse transcriptase activity; a neck mass from an American patient with Burkitt's lymphoma (BL) had a DNA polymerase activity which resembles the herpes-virus-induced DNA polymerase. The detection of this biochemical marker in a BL tissue provides further evidence for the involvement of herpesvirus in this malignancy. We continue to examine more samples from cancer patients for the viral DNA polymerases. We are growing several BL cell lines in culture; we have purified the EBV-DNA-polymerase from some of the virus-producing cell lines. Its properties are compared with those of DNA polymerases of other human herpesviruses. We noticed a correlation between the extent of EBV replication and level of the viral DNA polymerase in the cells; however, the level of cellular DNA polymerases decreased with an increase in the virus replication. In addition, the cellular DNA polymerases alpha, beta, gamma, and mitochondrial DNA polymerase, as well as terminal deoxynucleotidyl transferase (when present) have been purified from the human cancer samples for a qualitative and quantitative comparison with the polymerases of other eukaryotic systems. These polymerases have been further characterized and their distinguishing properties studied. We have been examining the mechanism of selectivity of certain newly synthesized nucleoside analogs such as (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdU) with a potent and selective antiherpesvirus activity by (a) comparing the effects of the corresponding triphosphates on activities of the cellular and viral DNA polymerases, and (b) by examining quantitative and qualitative changes in DNAs isolated from infected and uninfected cells grown in the presence of BVdU.

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205. DNA POLYMERASE(S) OF THE REPLICATION SV40 CHROMOSOMES

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We intend to identify and characterize the DNA polymerase(s) which participate in the replication of simian virus 40 (SV40) DNA. Similarities between SV40 DNA replication and cellular DNA replication suggest that the same DNA polymerase(s) carry out cellular DNA replication as well. Our approach is simple: We shall purify replicating SV40 chromosomes to the maximum extent possible from infected monkey cells, then dissociate DNA polymerase(s) from the replicating chromosomes, and then use standard techniques to compare the DNA polymerase(s) so obtained with DNA polymerases alpha, beta, and gamma prepared by conventional procedures from monkey cells. Because initial purification of replicating chromosomes allows us to start with much purer material than in standard polymerase purification procedures, we feel it is worthwhile to try to purify the replication polymerase(s) to homogeneity. By partial and complete reconstitution experiments, we shall try to determine how the replication polymerase(s) interact with other proteins of the replication complex, with DNA, and with each other. If more than one polymerase species is associated with replicating SV40 chromosomes, we shall try to determine the function of each polymerase species during the replication process.

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206. DNA POLYMERASES IN NORMAL AND NEOPLASTIC HUMAN CELLS

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The fundamental goal of my research program is the elucidation of the mechanisms involved in the coordination and control of DNA replication, chromosome segregation and cell division. Since it is a fundamental attribute of all living cells that there be rigorous coordination of chromosome replication and segregation to ensure precise equipartition of the genome at cytokinesis, it seems clear that the study of the mechanisms involved in this coordination is central to our understanding of the regulation of normal and abnormal cell growth. The present proposal is concerned with the continuation of our ongoing program of purification and characterization of the DNA polymerases from the human malignant cell line, KB. We have identified three distinct DNA polymerase activities in KB cells, two of which are associated with nucleus, and the third with the cytoplasmic fraction. We have expended our major effort to date on rigorous purification and characterization of these activities, and at the present time two of the enzymes appear to be homogeneous. We believe that only by working with homogeneous and rigorously characterized polymerase molecules will it be possible to investigate meaningfully structural and functional interrelationships among them and begin to explore how the regulation of their biosynthesis and intracellular distribution are related to the coordination and control of DNA synthesis in human cells. Morever, such studies provide an essential foundation for the understanding of alterations in DNA polymerase structure, function and intracellular distribution that may be associated with virus infection and transformation and with those alterations of normal cell growth regulation that are observed in hyperplastic and neoplastic states.

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207. THE STRUCTURE AND FUNCTION OF A DNA REPLICASE

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The overall objective of my research is to characterize the structure and function of components of the cell machinery responsible for DNA replication; the specific component to be examined in this project is the replication-specific enzyme, DNA polymerase III (pol III) of B. subtilis.

My approach includes: (1) the examination of enzyme structure through the use of novel, pol III-specific molecular probes and classical techniques of protein chemistry, and (2) the examination of the structure and function of pol III in replicative synthesis, using mutant enzymes and a bacteriophage system (SPP-1) which requires pol III to replicate its DNA.

The study of pol III structure, which will exploit homogeneous preparations of enzyme, will examine specifically: (1) the physical characteristics of the enzyme, its peptide map, content, antigenic nature, and structural features which may be common to replication-specific polymerases from other prokaryotic and eukaryotic systems; (2) the site of substrate binding, and (3) the site of reaction with the pol III-specific arylhydrazinopyrimidine inhibitors in an approach using wild-type and drug-resistant enzymes, irreversible inhibitor analogs, and peptide mapping.

The study of pol III function will exploit mutant enzymes and will examine specifically: (1) the role of the nuclease and polymerase activities of pol III in the editing and synthesis of DNA, (2) the nature of replication-specific forms of pol III and their interactions with other host-specific and virus-specific proteins required for replication of SPP-1 in vivo and in vitro.

208. INHIBITORS OF RNA-DIRECTED DNA POLYMERASE ON MOUSE LEUKEMIA AND BURKITT'S TUMOR

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This is part of a broader project. A summary of this subproject is not available.

209. REVERSE TRANSCRIPTASE AND DNA POLYMERASE IN LEUKEMIC CELLS

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This is part of a broader project. A summary of this subproject is not available.

210. ISOLATION OF DNA-UNWINDING ENZYMES FROM RAT LIVER

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To accomplish the semi-conservative replication of DNA and the segregation of daughter duplexes at cell division the parental DNA strands need to be separated and unwound. Because the separation of strands of the DNA helix is a thermodynamically unfavorable process, enzymes which denature DNA at the expense of ATP hydrolysis are strongly implicated in the process of DNA replication. I propose to do the following: 1) Isolate DNA-unwinding enzymes from rat liver by virtue of their single-stranded DNA-dependent ATPase activity. 2) Characterize the enzyme activities of binding and unwinding on various DNA substrates as a way of identifying the unwinding enzymes most likely involved in replication. 3) Construct novel DNA structures which imitate specific aspects of replicating DNA for use in the unwinding assays. One structure I plan to construct will provide an ideal substrate for testing the hypothesis that swivelase and unwinding enzyme are sufficient for separation and unwinding of topologically interlocked strands. This type of experimental approach will ultimately lead to the

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detailed understanding of the eukaryotic DNA replication complex.

211. RESEARCH ON DEOXYNUCLEOTIDYL TERMINAL TRANSFERASE (TDT) IN HUMAN LEUKEMIA

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OBJECTIVE: a) Classification of human leukemias by the determination of the presence of TdT; b) induction of differentiation in a human TdT positive lymphoblastoid cell line; c) the role played by TdT in DNA metabolism.

APPROACH: A) TdT was measured in lymphoid cells from peripheral blood and/or bone marrow of patients affected by different leukemias and in different phases of the disease. The enzyme was tested by two methods: 1) a biochemical assay using a cell free extract and based on the addition of labelled dGTP to the polymeric substrate d(pA)50; 2) a single indirect immunofluorescence method used on cell smears using rabbit anti-TdT antibodies kindly provided by Dr. F. J. Bollum. b) A human lymphoblastoid T-cell line (RPM1-8402), after treatment with some well-known inducers of differentiation (DMSO, Na-butyrate, retinoic acid, dimethylformamide and TPA) was tested for parameters of differentiation of the T pathway (TdT levels and E-rosette forming ability). c) To evaluate the possibility that TdT activity may be influenced by variations of dNTP pool, two human lymphoblastoid cell lines (RPM1-8402 and RPM1-8392) positive and negative for the enzyme were treated with different concentrations of nucleosides(d-G and thymidine). After a 24-hour treatment the cells were tested for DNA synthesis level and cell survival. The cell survival was also evaluated after nucleoside removal.

PROGRESS: a) Neoplastic cells from 47 patients with ALL at onset, 44 patients with ALL in remisson, 16 patients with ALL in relapse, 33 patients with AML at onset, 16 patients with CML in chronic phase and 18 patients with CML in blastic transformation were studied for the presence of TdT. TdT was detected in 33 patients with ALL at onset, in 4 patients with ALL in relapse, in 1 patient with AML at onset and in 6 patients with CML in blastic transformation. b) A human lymphoblastoid T-cell line (RPM1-8402) was induced to differentiate by a 96 hour treatment with TPA at concentrations of 10 to the minus 7th power and 10 to the minus 8th power M. After this period the level of TdT activity was reduced from 60 U/10 to the 8th power cells to 2.2 U/10 to the 8th power cells. (Text Abridged.)

212. BIOCHEMICAL EVENTS LEADING TO ALTERATION OF RNA AND DNA

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During the tenure of this grant, studies on the mechanism of action of a number of enzymes involved in procaryotic DNA replication will be continued. Particular emphasis will be placed on the mechanism of action of the DNA elongation enzymes involving E. coli DNA polymerase III, DNA elongation factors I, III and dna Z. Studies on the specificity of the action of dna G in the synthesis of oligonucleotide primers will be continued. We also plan to examine the mechanism of action of the phiX A protein and the control of phiX RFI replication.

Studies will continue on the mechanism of adenoviral DNA replication with particular emphasis on the enzymes involved in the elongation of this DNA by eucaryotic enzymes.

Studies will also be carried out on vaccinia viral transcription and the role of auxilliary proteins in transcription. Included in these studies will be an analysis of reactions occurring at ends of RNA chains. The latter projects will also be carried out with nuclear extracts of HeLa cells.

213. DNA GYRASE AND THE SUPERCOILING OF DNA

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This research is concerned with the mechanism and function of DNA gyrase. E. coli DNA gyrase will be purified to homogeneity using ion exchange and DNA affinity chromatography. The number of protein components required for activity will be determined by sodium dodecyl sulfate gel electrophoresis and ultracentrifugation.

A wide variety of physical organic techniques (including electron microscopy, mass spectrometry and restriction mapping) will be used to study a) the binding specificity of the enzyme on different DNA's, b) the mechanism of DNA nicking-closing catalyzed by DNA gyrase, and c) the role of ATP in the supercoiling of DNA catalyzed by DNA gyrase.

214. HUMAN LIVER APURINIC DNA ENDONUCLEASE

Springgate, C. F., Liu, L., Tulane University of Louisiana, School of Medicine, Dept. of Microbiology & Immunology, *1430 Tulane Ave., New Orleans, Louisiana*, 70112, United States of America

To investigate how human liver cells prevent or retard the malignant effects of chronic exposure to carcinogens present in the environment, the enzymology of the excision repair of DNA damaged by model environmental carcinogens will be investigated. In these initial studies a key DNA repair enzyme, human liver DNA endonuclease specific for apurinic/apyrimidinic sites (apurinic DNAse), will be studied. Preliminary studies have demonstrated that autopsy liver is a rich source of apurinic DNAse and have also suggested that the activity of apurinic DNAse in autopsy livers is fairly stable. In initial experiments, phosphocellulose chromotography has revealed multiple species of human liver apurinic DNAse. Each species of apurinic DNAse will be purified further by column chromotography on DNA cellulose and hydroxyapatite. The specificity of endonucleolytic incision by each enzyme will be examined by assaying incision with apurinic DNA, with apyrimidinic DNA, and with DNA damaged methyl methane sulfonate, by nitrogen mustard, by N-acetoxy-2-acetylaminofluorene, and by ultraviolet irradiation. Each purified apurinic DNAse will be tested for associated non-endonucleolytic activities including DNA exonuclease, DNA phosphatase, and DNA glycosidase. Taken together these studies should begin to define how human liver cells repair DNA damaged by environmental carcinogens

215. ENZYMATIC MODIFICATION OF NUCLEIC ACIDS

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We propose to continue our genetic and biochemical studies of the excision repair of uracil residues and of apurinic/apyrimidinic sites from microbial DNA and of the effects of this process on normal DNA replication, recombination, and mutagenesis. We shall study these processes in Escherichia coli mutants defective in one or more of the following enzymes: dUTPase, which prevents the misincorporation of uracil into DNA; uracil-DNA glycosylase, which removes uracil from DNA leaving an apyrimidinic site; and exonuclease III, which has an associated endonuclease activity for apurinic/apyrimidinic sites, and which is the major such endonuclease in E. coli and in Hemophilus influenzae.

For each of these enzymes, we shall continue to isolate and characterize tighter mutants (deletions and insertions) as well as overproducers (obtained by gene cloning techniques). We shall pursue further physicochemical and enzymatic studies of exonuclease III to see if the four activities of this enzyme are catalyzed by a common active site, and we shall also study the action of enzymatic reagents at apurinic/apyrimidinic sites in DNA.

216. CHROMOSOMAL PROTEINS FROM TUMOR CELLS

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OBJECTIVE: We will determine whether any of several nonhistone chromatin proteins that we have purified to apparent homogeneity are capable of stimulating the enzymatic activity of DNA-dependent DNA polymerase.

APPROACH: Individual nonhistone chromatin proteins will be purified from cultured rat hepatoma cells by salt extraction, ultracentrifugation, and ion-exchange chromatography. Nuclei isolated from the hepatoma cells will be incubated in the presence of the deoxynucleoside triphosphates, one of which

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will be radioactively labeled. DNA synthesis will be followed in the presence and absence of added nonhistone chromatin proteins by monitoring the incorporation of radioactivity into acid-insoluble material.

PROGRESS: Will examine the possible effect on DNA synthesis of individual nonhistone chromatin proteins from a line of cultured hepatoma cells (HTC cells). Current efforts are directed toward obtaining high and reproducible levels of DNA synthesis in isolated HTC cell nuclei and toward isolating individual nonhistones. The involvement of helix destabilizing proteins in DNA replication in prokaryotes suggests that such proteins might well be part of the DNA replication machinery in eukaryotes. Have begun to islolate potential, helix destabilizing proteins from NaCl-dissociated HTC cell nonhistone chromatin proteins by sequential chromatography on double-and single-stranded DNA columns. Results indicate that there are just a few HTC cell nonhistones that have preferential affinity for single-stranded DNA and which therefore might be helix destabilizing proteins. As individual proteins are purified will examine their effect on DNA synthesis in isolated nuclei.

217. ENZYMATIC MECHANISMS OF DNA SYNTHESIS

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The aim of this project is to understand more fully the process of DNA replication through detailed study of the mechanisms of selected DNA polymerases and factors which affect these enzymes. Experiments will concentrate on measurement of basic parameters of the enzyme mechanism in vitro, specifically including processivity, i.e., the number of nucleotides added each time the polymerase binds DNA. We have recently developed a quantitative assay for measurement of processivity of DNA synthesis in vitro (Bambara et al. (1978), J. Biol. Chem. 253, 413).

The availability of this assay provides an opportunity to examine factors which perturb DNA polymerases, specifically DNA replication-associated proteins. It is the major goal of this project to use such kinetic assays to detect and isolate DNA replication associated proteins in cell and viral systems where genetic tools are not available for this purpose. We will therefore begin a search of calf thymus cell extracts for proteins that increase the extent of processive DNA synthesis by purified calf alpha DNA polymerase. We will also search by the same means for stimulatory proteins related to the avian myeloblastosis virus (AMV) DNA polymerase.

BIBLIOGRAPHIC REFERENCES: Mechanism of Inhibition of the Avian Myeloblastosis Virus DNA Polymerase by Adriamycin. Steven W. Matson, Philip J. Fay and Robert A. Bambara, Biochemistry 19, 2089-2096, 1980. Kinetic Characteristics which Distinguish Forms of Calf Thymus DNA Polymerase Alpha. Robert A. Bambara and Joel W. Hockensmith, J. Supramolecular Struct. Abs., Supplement 4 (Ed. C. F. Fox, Alan R. Liss, Inc., New York), 1980.

218. CANCER BIOLOGY RESEARCH CENTER

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During the past year detailed studies on DNA gyrase have continued resulting in further specification on the mechanism of DNA binding and cutting, the development of in vitro system for the study of N4 replication and preparation of recombinant plasmids containing bacteriophage lambda stick ends (cosmids). Yeast mutants particularly useful for the study of recombination have been isolated and studied and a mechanism for the non-reciprocal transfer of information from one portion of a chromosome to another portion of the same chromosome has been discovered. Proteins binding to 5 and 5.8 S ribosomal RNA and initiator and elongator tRNA's have been identified. Differences between the site of inhibition of different DNA polymerase acting on carcinogen-reacted DNA templates have been observed.

219. THYMIDINE KINASE IN DNA SYNTHESIS OF MALIGNANCY (HUMAN, RATS)

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This is part of a broader project. A summary of this subproject is not available.

F. REGULATION OF DNA SYNTHESIS

220. PROPHYLAXIS OF SPONTANEOUS MAMMARY TUMORIGENESIS IN RATS BY A TEMPORAL INHIBITION OF MAMMARY DNA SYNTHESIS DURING YOUNG AGES

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OBJECTIVE: In cyclic rats, mammary gland DNA synthesis, which is a limiting factor for mammary tumorigenesis, is generally high during young ages and declines with the advance of age. The synthesis is mostly dependent upon prolactin and is therefore modulated by CB-154. This study was started to examine whether temporal inhibition of mammary gland DNA synthesis during young ages through suppression of pituitary prolactin secretion would result in the prevention of spontaneous mammary tumorigenesis in rats at advanced ages as a possible step to find out an efficient way for prophylaxis of human breast cancer.

APPROACH: Sprague-Dawley virgin rats were divided into 4 groups. Groups I and III received s.c. injection of CB-154 (0.5 mg) for 7 weeks beginning at 4 and 11 weeks of age, respectively, and Groups II and IV were given vehicle only as respective controls. Each rat is checked for palpable mammary tumors during its lifetime.

PROGRESS: Mammary tumor incidence until 20 months of age was almost completely inhibited (3%), while the incidence for the controls was 47%.

BIBLIOGRAPHIC REFERENCES: Nagasawa, H.: IRCS Med. Sci. 5: 405, 1977. Nagasawa et al.: Cancer Res. 36: 2223, 1976. Nagasawa, H. & Vorherr, H.: Amer. J. Obst. Gynecol. 127: 590, 1977. Nagasawa, H.: Med. Hypoth. 5: 1117, 1979.

221. EFFECTS OF ESTRADIOL AND ESTRIOL ON PITUITARY PROLACTIN SECRETION AND MAMMARY GLAND DNA SYNTHESIS OF RATS (ABBREV)

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OBJECTIVE: Estriol is often considered to be a weak estrogen and to act protectively on the development and progression of human breast cancer. This study was conducted to compare the effects of estradiol and estriol on pituitary prolactin secretion and mammary gland DNA synthesis, both being primary factors for mammary tumorigenesis, as a possible step toward evaluating the role of estriol in mammary tumorigenesis.

APPROACH: Ovariectomized female Sprague-Dawley rats were given daily s.c. injections of 5 or 50 micrograms of estradiol-17beta or estriol for 3 days, or received s.c. implantation of cholesterol pellets containing estradiol or estriol at a ratio of 1:10 for 48 or 72 hrs. Serum prolactin level as an index of the rate of pituitary prolactin secretion was assayed by radioimmunoassay and mammary gland DNA synthesis was estimated by the in vitro incorporation of (3H) thymidine.

PROGRESS: While daily injection of 50 micrograms of estriol increased serum prolactin levels, the effect was less than that of estradiol, and it did not promote mammary DNA synthesis. The administration of estriol in the pellet was effective on both factors to a similar extent as for estradiol, indicating that the different estrogenic effects of estradiol and estriol are mainly ascribed to the difference in the period of effective distal retention of the hormones and that a protective effect of estriol itself on breast cancer is unlikely.

BIBLIOGRAPHIC REFERENCE: Yanai, R. and Nagasawa, H.: Europ. J. Cancer 16:339, 1980.

222. REGULATION OF NUCLEIC ACID AND PROTEIN SYNTHESIS - A BACKGROUND STUDY RELATED TO THE BIOLOGICAL EFFECTS OF RADIATION

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This proposed program is an effort to uncover regulators of protein synthesis in living systems. It is hoped that a deeper

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understanding of the mechanisms by which such regulation is accomplished in normal cells will lead to a better perception of the aberrations which lead to the deficient protein synthesis associated with radiation damage, and the excessive protein synthesis of neoplasia. A particular dinucleotide (AP4A) has been discovered which is associated with rapid cell growth, and an effort is being made to define its function. A triadecamer deoxynucleotide has also been synthesized which may inhibit oncogenic viral replication.

223. AVIAN RETROVIRUS DNA SYNTHESIS AND ITS REGULATION

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The objective of this research program is to investigate the mechanisms involved in avian retrovirus proviral DNA synthesis and its regulation. The basic approaches involve characterization of the avian retrovirus DNA polymerase and its proteolytic cleavage products found in isolated virions or infected cells. Techniques utilized are peptide mapping; amino acid sequence analysis; specific immunoprecipitation techniques; and nucleic acid enzymology. We will try to define the role of each enzymatic activity, associated with each individual polymerase subunit and its proteolytic cleavage products, in proviral DNA synthesis. Major emphasis will be directed towards studying the newly discovered DNA endonuclease activity associated with the avian retrovirus alpha beta DNA polymerase and p32pol gene product.

224. REGULATION OF DNA SYNTHESIS IN THE NOVIKOFF HEPATOMA

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The overall objectives of this project are to identify proteins which regulate and/or participate in DNA replication and/or repair using the Novikoff hepatoma as a model system for eukaryotic DNA synthesis. The DNA polymerases have been purified and subsequently used to screen for proteins which stimulate the in vitro reaction. Several factors have been identified and purified to varying degrees. A double-strand specific exonuclease, mammalian DNase V, has been purified to homogeneity and characterized. A novel DNA-binding protein has also been found. Each of these factors is being characterized as individual proteins and then with respect to its interaction with the cellular DNA polymerases. The goal is to establish a role for each action with the cellular DNA polymerases. The goal is to establish a role for each in DNA synthesis in vivo, and to construct multienzymes complexes for DNA replication or repair in vitro.

BIBLIOGRAPHIC REFERENCES: Meyer, R.R. and D.W. Mosbaugh (1980), Characterization of mammalian DNase V, a double-strand exonuclease which interacts with DNA polymerase-beta of the Novikoff hepatoma, ICN-UCLA Symp. Mol. Cell. Biol., 19, 639-648. Small, P.K., D.C. Rein and R.R. Meyer (1981), An in vitro system for repair of DNA damage using purified proteins, J. Supramol. Struct. Cell. Biochem., suppl. 5, 178 (abstract).

225. DNA SYNTHESIS IN MAMMALIAN CELLS

Wilson, S. H., Planck, S., Detera, S., Becerra, P., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biosynthesis Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Two mammalian proteins important for analysis of in vitro DNA replication have been characterized. These proteins are DNA alpha-polymerase and helix-destabilizing protein-1 from mouse myeloma. Discrete fragments of the helix-destabilizing protein were produced by controlled proteolysis with trypsin. These fragments were purified and used in studies on structure-function relationships. We found that the N-terminal region of native helix-destabilizing protein-1 is not required for DNA binding and that DNA binding by the native protein occurs without significant protein:protein cooperativity. The kinetic mechanism of alpha-polymerase was studied using a simplified replication system in which the enzyme acted in a strictly processive fashion. The experimental results were consistent with a rapid equilibrium-random Bi Uni Uni Bi ping pong mechanism. It was also found that the initiation phase of the alpha polymerase reaction could be studied independently of the elongation phase.

BIBLIOGRAPHIC REFERENCES: Planck, S.R., Tanabe, K., and Wilson, S.H.: Distinction Between Mouse DNA Polymerases alpha and beta by Tryptic Peptide Mapping. Nuc. Acids. Res. 8, 1980. Planck, S.R., and Wilson, S.H.: Studies on the structure of Mouse Helix-Destabilizing Protein-1: DNA Binding and Controlled Proteolysis with Trypsin. J. Biol. Chem., in press, 1980.

226. REGULATION OF DNA SYNTHESIS IN DIPLOID AND TRANSFORMED CELLS

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Differences in DNA replication in diploid and transformed human cells were studied. Transformed cells lacked the capacity to discriminate against the incorporation of 5-bromodeoxyuridine into DNA in the presence of thymidine, because 1) they are defective in the excision of misincorporated bases and/or 2) different DNA polymerases are used. Repair synthesis induced by UV or MNNG was inhibited more effectively by novobiocin or nalidixic acid than either replicative synthesis or repair synthesis induced by methylmethane-sulfonate (MMS), suggesting that topoisomerase-mediated alteration of higher order DNA structure is required for DNA repair. However, cells could tolerate the disruption (by topoisomerase inhibitors) of such higher-order DNA structure for up to 24 hours and resume repair synthesis after UV. Replicative DNA synthesis in tumor cells defective in the repair of alkylation damage (Mer minus) was far more sensitive to MNNG than that in repair-proficient lines (Mer plus). Both Mer plus and Mer minus cells could incise DNA after MNNG treatment, but the Mer minus cells did so more slowly and did not restore their original DNA conformation, even after 24 hours.

227. DNA SYNTHESIS - REGULATION IN NORMAL AND CANCER CELLS

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The objective of the proposed research project is to define possible controls for DNA biosynthesis during normal and abnormal growth. 1) The interactions of enzymic and non-enzymic factors in the elongation step of DNA replication will be studied. We will characterize these components of the DNA synthesizing machinery and determine their mechanism(s) of action. 2) The basis for the molecular heterogeneity of DNA polymerase alpha in mammalian cells will be investigated. We will ascertain the possible physiological significance of this heterogeneity. 3) The relationship of chromatin associated to non-chromatin associated DNA polymerase alpha will be studied. We will ascertain if DNA polymerase alpha is translocated to the nucleus as a pro-enzyme.

228. DNA SYNTHESIS AND CELL DIVISION OF ESCHERICHIA COLI

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The genetic and biochemical study of DNA synthesis and cell division in Escherichia coli is the interest of our laboratory. Replication of the E. coli chromosome, the single most important controlling step in the cell cycle, is a complex and not completely understood process. Much of our knowledge of this process has been gained by the use of temperature-sensitive (ts) mutants defective in DNA synthesis. Physiological studies and studies of ts mutants have defined three major steps in replication: initiation, polymerization, and termination. The polymerization phase requires functional products of the dnaB, C, E, G, X, Y, Z, nalA, cou, and ssb genes, at least three additional proteins identified by biochemical studies, and possibly the supS gene. The genes dnaX, Y, and Z, and the supS mutation were identified in this lab. We propose to complete the characteriza-

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tion of the supS gene and determine the function of the wild-type gene, to complete the genetic characterization of the dnaX-Z region, to define the dnaX function, and to identify the function of the dnaY gene. Genetic studies will be facilitated by the use of complementation by transducing phages formed in vivo and by hybrid phages and plamids constructed in vitro and by the use of DNA sequencing. Products of dnaX and Y and possibly of supS will be identified by analysis of phage DNA replication inhibition at non-permissive temperature in vivo and by the phage SS-RF complementation assay in vitro. In addition studies on transcription of the dnaX-Z region will be begun. The DNA of transducing phages will be used to characterize mRNAs specific for the dnaX-Z region and to study the possible organization of these two genes into one functional unit.

229. REGULATION OF MITOCHONDRIAL DNA REPLICATION

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The proposed study will elucidate regulatory controls in the replication of mitochondrial DNAs. Replicative forms will be isolated from thymidine kinase-minus lines of mouse and human cells. A displacement loop model for human mitochondrial DNA replication will be established and compared to that previously constructed for mouse mitochondrial DNA. Control of replication at the level of transcription will be examined through the use of dark-field electron microscopy of transcription complexes and by density gradient and velocity centrifugation of pulse and pulse-chase labeled components. Regulation mediated through mitochondrial specific DNA nucleases in displacement replication will be detailed. Altered replicative forms produced in the stimulation of mitochondrial DNA synthesis by infection of sensitive cells with oncogenic viruses will be examined. The sites on mouse and human mitochondrial DNA where regulatory elements function will be determined relative to known mitochondrial genes and secondary structures by techniques of electron microscopy, gel electrophoresis and thermal denaturation of restriction endonuclease and Alteromonas endonuclease fragments.

Parallel studies will be conducted with circular oligomer and catenated forms of mitochondrial DNA purified from human leukemic white cells before and after patients are treated with antileukemic drugs. Examination of replicative forms will permit construction of a model for displacement replication of human circular dimer mitochondrial DNA. Intramitochondrial distributions of catenated monomeric and oligomeric forms may reflect spatial disposition and relative activities of DNA nucleases in leukemic cell mitochondrial DNA and will be assessed by electron microscopy of topological configurations differentially contrasted by vacuum disposition of heavy metals. Altered mitochondrial ultrastructures in human leukemic myeloblasts will be correlated with frequencies of complex mitochondrial DNAs.

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230. CONTROL OF DNA REPLICATION IN NORMAL AND TUMOR CELLS

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This proposal has as its objective an investigation of control of growth and DNA replication in normal and neoplastic tissues. Four major approaches will be used. First, we intend to purify and characterize DNA polymerases present in the nucleus. Activities as well as physical properties of the DNA polymerases from liver nuclei and hepatoma nuclei will be compared. Any differences observed between the two tissues will be investigated in a fast growing, an intermediate growing and a slow growing hepatoma. Secondly, we intend to identify, separate and, hopefully, reconstruct nuclear components that make up a replicating complex. It is by now obvious that factors other than DNA and DNA polymerase must be part of the replicating system. We will look for such factors in nuclei and investigate their possible significance in the control of DNA replication. Thirdly, we will use bleomycin to investigate possible differences in DNA repair in liver and hepatoma cells. Bleomycin stimulates 3H-TTP incorporation in a nuclear incorporating system. Stimulation of host liver nuclei is much more dramatic than stimulation of hepatoma nuclei. The possibility that this difference is due to a less efficient repair system in hepatomas will be explored. Fourthly, the effect of cytoplasmic or humoral factors on DNA replication will be investigated. There is evidence that such factors play a role in control of growth and DNA synthesis. By using factors from hepatoma tissues or tumor-bearing animals on nuclei or cells of liver, or factors from normal animals on nuclei or cells from hepatomas, we hope to obtain evidence that such factors might influence the loss of control in neoplastic cells.

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231. STUDIES ON DNA REPLICATION AND RECOMBINATION

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Nucleic acid biochemistry: We are currently involved in three lines of experimentation that deal with the biology and chemistry of nucleic acids. 1. The first line of experimentation deals with the isolation and characterization of the regulatory DNA elements which serve as the origins for DNA synthesis in the viruses phi X and G4.

By a combination of biophysical and electron microscopic techniques we can locate the regulatory sequences at which the synthesis of positive strand DNA is initiated to form rolling circle intermediates, and the point at which negative strand DNA is initiated to convert single-stranded circles to duplex rings. The latter initiation event represents the reaction leading to the formation of an Okazaki fragment. 2. A second line of experimentation involves the isolation and sequencing of the three DNA regions in bacteriophage phi-X which function as sites for the initiation of messenger RNA synthesis. 3. Our most extensive current effort involves the study of DNA recombination. We have been able to use the electron microscope to observe individual DNA molecules and analyze intermediates in the process of recombination.

Current recombination experiments under way include a) the observation of recombination intermediates in a eukaryotic system, specifically SV40 infected cells, b) the analysis of a mutation (the chi mutation) that creates hot spots for genetic recombination, and c) the development of an in vitro system for studying the enzymology of generalized recombination.

232. PROBES OF THE ORIGIN OF MITOCHONDRIAL DNA REPLICATION

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Previous studies in this laboratory have shown that the structure and synthesis of mitochondrial DNA (mtDNA) in normal and malignant animal cells is intimately related to the mitochondrial membrane structure. The two origins/termini of asymmetric hamster mitochondrial DNA replication have been mapped precisely in a detailed restriction map. Little is presently known about regulation of mtDNA replication. The objective of this study is to determine in detail the structure and location of possible controlling elements in the regions of the two origins of mtDNA replication. It will be investigated at several related but distinct levels of organization: (1) DNA-membrane association; (2) DNA-protein interaction; (3) DNA replication in rela-

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tion to membrane and/or protein structure; (4) DNA secondary structure; and (5) nucleotide sequence organization.

233. INITIATION OF DNA REPLICATION IN TUMOR VIRUS CHROMOSOMES

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Our long-term goal is to understand, at the molecular level, how mammalian cells replicate their chromosomes and how chromosome replication is related to the control of cell proliferation. In this proposal, we utilize Simian Virus 40 (SV40) chromosomes as a simple, but appropriate, model system for mammalian chromosomes that is well suited for studies requiring a detailed analysis of DNA replication, DNA sequence and chromatin structure. We have four (4) specific aims directed at understanding the mechanism for initiation of DNA synthesis and the significance of nucleosome arrangement with respect to DNA sequence (nucleosome phasing). (1) Map the locations and analyze the DNA sequence of specific DNA sites recently found throughout the SV40 genome that promote initiation of Okazaki fragments on native replicating chromosomes, and relate these data to the metabolism of RNA-primed nascent DNA chains (Okazaki fragments). (2) Determine the precise nucleotide locations of 5'-end(s) of nascent DNA chains at the unique origin of SV40 DNA replication. Relate the origin DNA sequence thus defined to the Okazaki fragment initiation sites, the binding sites for T-antigen, and the structure of RNA-primed nascent DNA chains. These results will be used to design a specific assay for initiation of DNA replication in vitro. (3) Map, sequence and analyze genomic locations of nucleosomes on native SV40 chromosomes in order to provide a histogram of the relative number of nucleosomes at a given genomic position, and relate nucleosome locations to DNA sequence signals for DNA replication and transcription. DNA sequences that direct initiation or termination of DNA replication or transcription may be specified by nucleosome phasing. (4) Develop a subcellular system that will faithfully reproduce initiation of SV40 DNA replication. These experiments will accurately define what constitutes DNA synthesis initiation signals and relate our comparatively extensive knowledge of events at active replication forks to the problem of initiation of these events at the origin of replication. Once the involvement of DNA sequences at various steps in DNA replication is understood, then the availability of subcellular systems that accurately carry out the sequence of events in initiation of replication will permit a detailed biochemical investigation.

234. CHARACTERIZATION OF MAMMALIAN ORIGINS OF DNA REPLICATION

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I plan to study the control of DNA replication in mammalian cells by examining the structure, organization, and function of sites where replication is initiated in chromosomal DNA. Using 'libraries' of mouse or human DNA fragments cloned in a prokaryotic vector, I will screen for genomic sequences which act as origins of replication when introduced into mammalian cells. A high transfection efficiency will serve as a selective criterion for sequences able to replicate autonomously. Similar screening procedures will be performed on previously cloned portions of mouse and human genomes containing immunoglobulin genes. As a complementary approach to the isolation of chromosomal origins of replication, I will screen libraries or isolated genomic clones for sequences which hybridize to the SV40 origin of replication. Such sequences will be tested for function as replication origins when introduced into mammalian cells as part of an appropriated cloning vector. Structural and functional studies will be undertaken to determine whether specific classes of replication origins exist in mammalian DNA. Functional and organizational alterations in replication origins will be examined in association with DNA rearrangements involved in immunoglobulin gene differentiation.

235. ENZYMATIC DNA MODIFICATION IN CELL DETERMINATION AND IN THE CONTROL OF NORMAL AND NEOPLASTIC GROWTH

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OBJECTIVE: The aim of this project is to study whether nuclear enzymatic DNA modifications are related to oncogenic phenomena. This idea is based on the 'synchron model' which hypothesizes DNA modifying enzyme-caused heritable changes underlie ontogenesis and carcinogenesis, in higher eukaryotes. The synchron model predicts that enzymatic DNA modifications, some of them probably occurring via a two-step mechanism, methylation and aminohydrolysis of 5-methyl-cytosine at the DNA level, regulate the cell lineage of higher eukaryotes.

In the last two years several authors have reported data in agreement with some aspects of the synchron model. (Razin A. and Riggs A.D. (1980) - Science 210, 604-610.) Furthermore, heritable changes in the immunoglobulin genes have been reported to take place during the terminal differentiation of lymphocytes.

Environmental or genetic factors might cause similar events to occur in the differentiated cells of adult organisms. The appearance of specific DNA modifying enzyme might cause a normal cell to undergo heritable changes and become a cancer cell.

APPROACH: So far we have tested the effect of anticancer agents on the DNA methylase activity present during sea urchin and amphibian embryo early development. The state of methylation of specific genes may be studied with restriction endonucleases and this methodology will be used wherever possible.

BIBLIOGRAPHIC REFERENCES: Tosi, L. & Scarano, E. (1979). Macromolecules in the Functioning Cell (Eds. F. Salvatore, C. Marino & P. Volpe), Plenum Publishing Corp., New York, p. 239-253.

236. CONTROL OF DNA SYNTHESIS - EFFECT OF ANTITUMOR ANTHRACYCLINE DRUGS ON PROTEINS AND ENZYMES WHICH AFFECT THE CONFORMATION OF DNA

Gambetta, R. A., Zunino, F., Velcich, A., Istituto Nazionale per lo Studio e la Cura dei Tumori, Dept. of Oncologia Sperimentale, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: The objective is to define the details of the mechanism of DNA synthesis inhibition by anthracycline antibiotics.

APPROACH: Several DNA specific proteins and enzymes will be isolated from mammalian sources and the interference of anthracyclines in the activity of these proteins on DNA will be determined.

237. ENZYMOLOGY OF SV40 DNA REPLICATION - IN VITRO STUDIES

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Although our current knowledge of mammalian DNA replication suggests that many enzymes and other proteins are involved, none have until recently been shown to be required. To determine which enzymes are required for DNA replication, I propose to analyze the replication in vitro of simian virus 40 (SV40) chromosomes. Replicating SV40 chromosomes can be isolated in a form which allows DNA synthesis to continue. The relative simplicity of this system should enable us to fractionate the enzymes bound to these chromosomes and determine their role(s) in DNA replication. The chromosomes will be assayed for the presence of enzymes believed to be involved in replication. For each enzyme detected, we will determine whether it is preferentially associated with replicating SV40 chromosomes. We will also examine whether the removal or inhibition of each enzyme results in an alteration in the process of DNA replication. If an alteration is detected it provides excellent evidence for the role(s) of that enzyme in the replica-

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tion process; if no effect is found, the possibility of adventitious association will be tested.

BIBLIOGRAPHIC REFERENCES: Edenberg, Howard J. (1980) Extraction of transcribing SV40 chromosomes in very low salt. Nucleic Acids Research, 8, 573-586. Edenberg, Howard J. (1980) RNA- and DNA- polymerases associated with SV40 chromosomes. J. Supermolecular Structure, Supplement 4, p.334.

238. ROLE OF DNA RELAXING ENZYME IN SV40 DNA REPLICATION

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The proposed research seeks to determine the involvement of the DNA relaxing enzyme in simian virus 40 (SV40) and eukaryotic DNA replication. SV40 DNA replication can serve as a model for cellular DNA replication since, like cellular DNA, SV40 DNA is replicated in the nucleus by cellular enzymes and is associated with cellular histones to form the characteristic nucleosome structure.

SV40 chromosomes isolated at low ionic strength from infected monkey cells retain the ability to replicate DNA in vitro when supplied with deoxy- and ribonucleoside triphoshates. Thus, at least some of the enzymes which participate in in vivo DNA replication must be bound to the replicating chromosome (RC). The DNA relaxing enzyme cosediments with RC (and also with non-RC) on neutral glycerol gradients and thus may be bound to RC and participate in their replication. Attempts to determine if the enzyme is bound by further purifying the RC have thus far been unsuccessful.

As an alternative approach, the requirement of the enzyme for in vitro SV40 DNA synthesis will be tested after addition of either a specific inhibitor (if one can be found) or anti-relaxing enzyme antibodies (if they can be raised towards the purified enzyme).

A 125,000 molecular weight form of the enzyme has been purified from nuclei of bovine liver in the presence of protease inhibitor. The previously characterized enzyme of approximately 64,000 molecular weight appears to be an active proteolytic fragment of the larger species. Attempts are being made to eliminate the variable proteolytic degradation and to isolate and characterize the intact enzyme.

BIBLIOGRAPHIC REFERENCES: Kowalski, D., Tricoli, J.V. and Sanford, J.P. (1980) Purification and Characterization of, and Fluorescence Spot Test for, DNA Nicking-Closing Enzyme from Bovine Liver, 9th Ann. ICN-UCLA Symp., J. Supramolec. Struct., Suppl. 4 (abst. 903), 336. Kowalski, D. and Sanford, J.P. (1980) Specific Single-Strand Cleavages Produced by Mung Bean Nuclease in Superhelical PM2 DNA Near pH 7, Fed. Proc. 39, (Abst, 1411), 1875.

239. REGULATION OF RECA GENE EXPRESSION

Echols, H., Crowl, R. M., University of California, Berkeley Campus, School of Letters & Science, Dept. of Molecular Biology, *Berkeley, California,* 94720, United States of America

Existing evidence indicates that neoplastic transformation induced by physical and chemical carcinogens is initiated by DNA damage. In E. coli, DNA damage elicits a number of cellular responses including mutagenesis and prophage induction. Derepression of the recA gene seems to be the key event leading to the expression of these responses. The objective of this research program is to define the molecular mechanism involved in the regulation of recA gene expression. Achievement of this objective will require the isolation of the regulatory protein for recA and the identification of the inducing signal.

The general approach for detecting the regulatory protein will be to search for a binding activity which binds to lambda recA srlC DNA, but not to lambda srlC DNA. Methods used to isolate other DNA binding proteins will be employed to develop a purification scheme for the recA DNA-specific binding activity. Experiments are planned to study the mode of inactivation of the binding activity in the cell following inducing treatments. Reproduction of this process in vitro may provide an approach for the identification of the inducing signal.

240. STRUCTURE A REPLICATION OF SPECIFIC EUKARYOTIC GENES STUDIED USING RECOMBINANT DNA TECHNIQUES

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OBJECTIVE: The objective of this project is to study the molecular mechanism of DNA replication and gene amplification and the mechanisms involved in the control of DNA replication in a eukaryotic organism.

APPROACH: The approach to this problem is to study the replication in vitro of a specific gene from a eukaryotic organism. The rDNA from Tetrahymena is an autonomously replicating episome-like molecule in the macronucleus of the organism. It is a giant palindrome and has a rotational axis of twofold symmetry. It is easy to isolate and its replication will be studied using enzymes from Tetrahymena to try to define the function of the components catalyzing the replication of a specific gene. Specificity of replication will be assayed by restriction endonuclease analysis of products and by direct visualization of products with the electron microscope. The origin of replication will be isolated and cloned onto plasmids and its nucleotide sequence determined. Amplification of rDNA will also be studied and the mechanism of rDNA reorganization during the sexual cycle in Tetrahymena will be investigated using recombinant DNA techniques.

241. CARCINOGENS ON PRO- AND EUCARYOTIC DNA REPLICATION

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The elucidation of the enzymes involved in and the mechanism of adenovirus DNA replication is continuing. We have been able to isolate five protein fractions necessary for Ad-DNAx replication. These fractions are being characterized and a search for new ones is continuing. The initiation reaction of Ad-DNA replication in which an Ad-DNA protein complex if formed is being examined and the components essential for this are being isolated.

Studies on the mechanism of control of the initiation of phi X RFI DNA replication are continuing. The nature of the ligase-type activity of the phi X gene A protein is being examined utilizing a plasmid which contains two gene A interaction sites as a template for in vitro DNA replication. The nature of lagging strand synthesis in these plasmids is being examined.

The influence of various carcinogens on each of these DNA replication systems will be examined.

BIBLIOGRAPHIC REFERENCES: Tertiary Structure is Involved in the Initiation of DNA Synthesis by the dnaG Protein. E.W. Benz, Jr., J. Sims, D. Dressler and J. Hurwitz. I.C.N. Winter Symposium on Mechanistic Studies of DNA Replication and Genetic Recombination; Bruce Alberts, ed., 1980, in press. DNA Research Updated. J. Hurwitz, Science 208, 723 (1980).

242. NATURAL AND ARTIFICIAL CONTROL OF DNA FUNCTION

Crothers, D. M., Dattagupta, N., Chaires, J. B., Yale University, Graduate School, Dept. of Chemistry, *206 Elm, New Haven, Connecticut,* 06520, United States of America

The purpose of this work is to elucidate the interaction of drugs with DNA and chromatin, and to prepare new polymeric drugs with increased specificity for binding to particular DNA sequences.

BIBLIOGRAPHIC REFERENCES: Selective Repression of Transcription by Base Sequence Specific Synthetic Polymers, L.D. Kosturko, N. Dattagupta & D.M. Crothers, Biochemistry 18 5751-5756 (1979). Unfolding of Nucleosomes by Ethidium Binding, H.M. Wu, N. Dattagupta, M. Hogan & D.M. Crothers, Biochemistry 19, 626-634 (1980).

243. THE ROLE OF DNA CONFORMATIONS IN GENE REGULATION AND DNA-PROTEIN INTERACTIONS

Singleton, C. K., University of Wisconsin, Madison Campus, School of Agriculture & Life Sciences, Dept. of Biochemistry, *Agriculture Hall, Madison, Wisconsin,* 53706, United States of America

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The proposed research will involve studying the role of DNA conformations in gene regulation. Through several lines of evidence, secondary and tertiary structure of DNA has been implicated as being involved in gene regulation via DNA-protein recognition and interaction. The aim of this project is to investigate in biochemical and physical terms how regulatory proteins and nucleic acids enzymes interact with specific regions of DNA. Studies involving E. coli lac operator, phage lambda operator, various promoters from E. coli and phages lambda and T7, replication initiation sites, and other regulatory regions will be carried out. Investigations to determine the intrinsic conformational properties of such regions as well as changes in these properties upon interaction with neighboring DNA regions and specific proteins will be done. Methods of investigation will include sedimentation and viscosity measurements, viscoelastic studies, circular dichroism, ultraviolet spectroscopy, high resolution thermal denaturation studies, sequencing, and DNA-DNA and DNA-protein crosslinking experiments.

244. BIOCHEMICAL STUDIES ON THE REGULATION OF DNA FUNCTION

Bode, V. C., Kansas State University, School of Arts & Sciences, Dept. of Biological Sciences, *Anderson Hall, Manhattan, Kansas*, 66502, United States of America

Experiments are planned with early mouse embryos and cell lines derived from them to study the control of alpha-fetoprotein synthesis. In vitro fertilization using tw5 sperm will be used to study the altered transmission ratio exhibited by mutants that are heterozygous for the tw5 region. A study of the retinoic acid-induced embryonal carcinoma cell differentiation will be continued.

BIBLIOGRAPHIC REFERENCES: Bode, V.C. (1980). Hugger, a new neurological mutant of the mouse. Genetics 94, in press (abstract). Bode, V.C. (1980). Retinoic acid induces every PC13 embryonal carcinoma cell to give rise to END cells. J. Supramol. Structure, Supplement 4, p. 215 (abstract).

245. REGULATION OF TRANSCRIPTION BY DNA-PROTEIN COMPLEXES

Gralla, J., University of California, Los Angeles Campus, Inst. of Molecular Biology, Dept. of Chemistry, *405 Hilgard Ave., Los Angeles, California*, 90024, United States of America

The project involves investigating the role of specific protein-DNA complexes in the regulation of gene expression. The principal focus is on the role of RNA polymerases and accessory protein factors in regulating the initiation of transcription. Particular attention is directed to the manner in which DNA sequence directs and mutation alters, transcription. Model templates include mutant E. coli lac promoters, and the genome of the eukaryotic simian virus 40. Proteins studied include E. coli RNA polymerase and lac repressor, RNA polymerase II from several eukaryotic sources, and chromatin-association protcins.

BIBLIOGRAPHIC REFERENCES: J. Stefano, J. Ackerson and J. Gralla: Alterations in two conserved regions of promoter sequence lead to altered rates of polymerase binding and levels of gene expression, Nuc. Acids Res., 8, 2709 (1980). D.W. Chandler and J. Gralla: Specific Binding and Protection of Form II SV40 Deoxyribonucleic Acid by Ribonuclei Acid Polymerase II from Wheat Germ, Biochemistry 19, 1604 (1980).

246. SHIFTS IN MAIZE CHROMATIN AND DNA FOR GENE ACTIVATION

Sheldon, E. L., University of California, Berkeley Campus, School of Letters & Science, Dept. of Genetics, *Berkeley, California*, 94720, United States of America

The objective is to find DNA or chromatin structural changes involved in gene regulation. The anaerobically structural changes involved in gene regulation. The anaerobically inducible maize Adh genes which code for alcohol dehydrogenase (ADH) will be cloned. Adh genes will be isolated from different tissues before and after induction by anaerobiosis. Restriction enzyme analysis and DNA sequencing will locate any changes in DNA primary structure. Active genes have been shown to be exposed in chromatin to DNAse I digestion, but inactive genes are usually protected from digestion (Weintraub and Groudine, 1976, Science 93, 848-858). The timing of the exposure of the Adh genes will be determined by digestion of chromatin from different maize organs before and after anerobic induction. ADH coding hybridization probes will be used to determine if the Adh genes are protected from digestion. Currently cDNA clones are being constructed from ADH coding mRNA for use as hybridization probes in the chromatin experiment and in cloning Adh genes. I have already made a recombinant DNA library which will enable the isolation of single copy maize genes (manuscript in prep.).

247. ARRANGEMENT AND REGULATION OF GENETIC INFORMATION IN ANIMAL CHROMOSOMES

Hogness, D. S., Stanford University, School of Medicine, Dept. of Biochemistry, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

The general objectives of this research are the elucidation of the molecular mechanisms of genetic regulation in animals, with particular reference to genes that control development in Drosophila melanogaster. The specific aims of the research supported by this grant are to define the DNA sequences and protein factors that control the accuracy and efficiency of in vitro transcription of D. melanogaster genes that we have isolated by molecular cloning. The genes that we are examining in this regard belong to the following four sets: (1) the histone genes, (2) members of a genetic regulatory hierarchy triggered by the steroid hormone, beta-ecdysone, (3) genes in the bithorax complex which determine the developmental pathways yielding certain of the body segments of this fly, and (4) the heat shock genes.

248. BIOCHEMICAL AND GENETIC STUDIES OF DNA SYNTHESIS

Yeh, Y., University of Arkansas, Medical Sciences Campus, School of Medicine, Dept. of Biochemistry, *4301 W. Markham St., Little Rock, Arkansas*, 72201, United States of America

The present project embraces the following aims: (1) To gain a general understanding of the biochemical and genetic mechanisms controlling the synthesis, repair and recombination of DNA. (2) To establish the function of genes that are essential for the continuation of DNA replication after it is initiated. (3) To elucidate the role of phage and host functions in the mechanism by which the monomer DNA of early T4 replicative stage is converted into the concotemer DNA of the late replicative stage. (4) To shed light on the mechanism by which growth factors initiate DNA synthesis in mammalian cells. (5) To comparatively study the control of deoxyribonucleotide biosynthesis in normal mammalian cells and their chemical transformed counterparts.

249. MECHANISM AND CONTROL OF DNA REPLICATION

Kornberg, A., Stanford University, School of Medicine, Dept. of Biochemistry, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

We propose to resolve and purify to homogeneity the near 20 proteins which make up the replication machinery of E. coli. The physical and functional properties of these proteins will be defined by using the small phage chromosomes as template probes. A complete, step-by-step, molecular description of the entire replicative life cycle of these phage DNAs should go a long way toward clarifying the mechanisms of fork movement of the replicating chromosome. With regard to the initiation (and termination) of a cycle of E. coli chromosome replication, the origin (oriC) will be identified, transferred to a high-copy-number plasmid and its functional features explored in enzymatic studies of replication of that hybrid plasmid. Progress in this direction may reveal some facts about the fine regulatory chemistry which governs the growth of cells, integration of chromosomal duplication and cell division, and factors which distort orderly growth to premature cessation or uncontrolled proliferation. Enzymologic, genetic and physicochemical methods will be applied to gain these chemical insights into one of the most basic biologic processes.

BIBLIOGRAPHIC REFERENCES: Shlomai, J. and Kornberg, A., An Escherichia coli replication protein that recognizes a unique sequence within a hairpin region in phi X174 DNA, Proc. Natl. Acad. Sci. USA 77, 799-803 (1980). Meyer, R. R., Glassberg, J., Scott, J. V. and Kornberg, A., A Temperature-sensitive single-stranded DNA-binding protein from Escherichia coli, J. Biol. Chem. 255, 2897-2901 (1980).

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250. STRUCTURE AND SYNTHESIS OF DNA

Englund, P. T., Johns Hopkins University, School of Medicine, Dept. of Physiological Chemistry, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

The summary for this project is under entry no. 50.

251. PROTEIN PHOSPHORYLATION AND CONTROL OF DNA SYNTHESIS

Miller, M. R., West Virginia University, School of Medicine, Dept. of Biochemistry, *Medical Ctr. Dr., Morgantown, West Virginia*, 26506, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

252. REGULATORY MECHANISMS OF DNA SYNTHESIS

Eberle, H., University of Rochester, School of Medicine & Dentistry, Dept. of Radiaton Biology & Biophysics, *601 Elmwood Ave., P.O. Box 607, Rochester, New York*, 14642, United States of America

This work contributes to the understanding of how DNA replication and cell division are regulated in bacterial and eukaryotic cells and how chemical, physical, and biological agents can lead to the alteration of the normal regulation of these processes. Work leading to understanding the mechanisms of regulation and to the isolation and characterization of regulatory sequences from normal (and eventually malignant) cells may allow a better understanding of the mechanism of carcinogenesis and lead to more sensitive or specific assays for the detection of carcinogenic agents.

253. ROLE OF SECONDARY STRUCTURE IN GENE REGULATION

Godson, G. N., Yale University, School of Medicine, Dept. of Therapeutic Radiology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

G. OTHER STUDIES OF DNA BIOCHEMISTRY

254. HYBRIDIZATION, DNA FUNCTION, MUTATION IN CELL CULTURE

Littlefield, J. W., Felix, J. S., Johns Hopkins University, School of Medicine, Dept. of Pediatrics, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

The mechanisms causing cell variability in differentiation, development and neoplasia can be examined using the methods of somatic cell genetics. Our goal is to obtain new information about these mechanisms by using embryonal carcinoma cells capable of determination and subsequent differentiation in vitro - an exciting surrogate for the mammalian embryo. Our objectives are: 1) to look for restoration of the ability to differentiate to endoderm among several nullipotent embryonal carcinoma cell lines through various modes of communication with pluripotent cells or other nullipotent lines; 2) to isolate endoderm-deficient variants from the pluripotent 'nursed' embronal carcinoma cell line PSA1 and from the 'emancipated' lines H6 and OC15S1; 3) to construct complementation groups among multiple variants within each of these lines using hybridization and perhaps cybridization techniques; and 4) to investigate the biological and biochemical nature of the defects in these complementation groups. With these studies we hope to construct eventually a complementation map of the biochemical steps involved in the commitment of pluripotent embryonic cells to endoderm and to identify these individual steps.

255. THE MECHANISM OF DNA UPTAKE AND GENETIC RECOMBINATION IN BACTERIAL TRANSFORMATION

Smith, H. O., Johns Hopkins University, School of Medicine, Dept. of Microbiology, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

One objective is to determine the mechanism of DNA uptake and genetic recombination in bacterial transformation. The organism for study is Haemophilus influenzae Rd, an efficiently transformable organism, that has been shown to recognize and take up only its own DNA from the medium. We are studying the mechanism of this specificity using a purified cloned fragment of Haemophilus DNA as donor. We have shown that short nucleotide sequences, present in Haemophilus DNA, are recognized by a membrane receptor protein during the initial stage of uptake. Our plans are to determine the base sequence of the DNA sites, purify and characterize the receptor protein, and study the kinetics of DNA transport.

Another objective is to study sequence-specific DNA-protein interactions using restriction and modification enzymes as model systems. We have cloned the HhaII restriction and modification genes from Haemophilus haemolyticus into E. coli to obtain enzyme overproduction. We plan to purify, characterize, and crytallize the two enzymes. We plan crystallographic studies of enzyme structure so as to determine how it fits the substrate site. We shall also determine binding and activity on chemically modified sites. Finally, the genes will be sequenced and their expession and control in E. coli examined.

256. DEVELOPMENT OF MAPPING PROCEDURES FOR REGIONS OF NUCLEIC ACID SECONDARY STRUCTURE TO SEQUENCE RESOLUTION

Hearst, J. E., University of California, Berkeley Campus, School of Chemistry, Dept. of Chemistry, *Berkeley, California*, 94720, United States of America

My laboratory has, in the past 3 years, developed a mapping procedure for major regions of secondary structure in single-stranded nucleic acids utilizing the psoralen photo-cross-linking reaction and electron microscopy. These techniques have detected both simple hairpins and large hairpin loops in fd DNA, SV40 DNA and 16S E. coli rRNA. The goal of this proposal is to improve these mapping techniques to the point where they can establish which bases in a nucleic acid sequence are base paired in these structural features. Experiments will concentrate on the fd DNA as a model system.

257. X-RAY DIFFRACTION STUDIES OF AN ECO RI RESTRICTION ENZYME-DNA COMPLEX

Graves, B. J., University of California, Berkeley Campus, School of Letters & Science, Dept. of Chemistry, *Berkeley, California*, 94720, United States of America

Specific recognition between DNA and proteins is one of the most fundamental processes in living cells, and plays a central role in diverse cellular events such as DNA replication, transcription, translation, repression, DNA restriction and modification. However, at present, we know very little about the detailed mechanism of such recognition processes at the atomic or structural level. Overall objective of this proposal is to determine the 3-dimensional structure of Eco RI restriction enzyme-DNA complex, one of the simplest protein-DNA recognition systems, by single crystal X-ray diffraction methods.

258. HUMAN LINKAGE MAPPING WITH DNA POLYMORPHISM

White, R. L., University of Massachusetts, Medical School at Worcester, School of Medicine, Dept. of Microbiology, *55 Lake Ave. N., Worcester, Massachusetts*, 01605, United States of America

We propose to develop a set of recombinant DNA probes to detect restriction fragment length polymorphisms in human DNA. Using human pedigrees we will construct a human linkage map. This linkage map can be used to examine and map possible genetic disease loci.

Our approach will use human recombinant DNA probes and restriction enzymes to define polymorphism by the method of Southern, which permits this analysis with a small sample of DNA from an individual. Using DNA from related individuals in pedigrees studied at U. of Utah by Dr. Mark Skolnick, we will determine linkage relationships.

259. SEQUENCE-SPECIFIC DNA-PROTEIN INTERACTION - RESTRICTION ENDONUCLEASES AS A MODEL SYSTEM

Mann, M. B., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Biochemistry, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas*, 77030, United States of America

The molecular cloning of bacterial restriction-modification (R-M) systems is proposed. A general method is to be developed, based on the original method which was used to isolate the HhaII R-M system from Haemophilus haemolyticus. The

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approach is a 'shotgun' method in which fragments of bacterial DNA are inserted into the plasmid vector, pBR322. Following transformation clones are selected by their ability to grow in the presence of a high titer of two or more virulent coliphages. Recombinant plasmid carrying R-M genes will be manipulated to create overproducer clones. This has been done for the HhaII R-M system using a thermoinducible plasmid. Ten mg of homogeneous HhaII restriction enzyme can be isolated from 100 g of cell paste. The protein will be used for X-ray diffraction analysis and for physicochemical and mechanistic studies.

A genetic approach will be developed using restriction endonucleases to investigate sequence-specific DNA-protein interaction. Conditional lethal (temperature sensitive phenotype) restriction enzyme mutants having the property of normal cleavage activity but with broadened specificity will be isolated following plasmid mutagenesis. Analysis of the site of several such mutations by DNA sequencing will reveal which amino acids are selectively involved with the DNA recognition ability of the endonuclease.

260. CONFORMATIONAL TRANSITIONS IN POLYPEPTIDES AND DNA

Jernigan, R. L., Miyazawa, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Mathematical Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose is to elicit molecular details of conformational kinetics in polypeptides and DNA. An eventual aim is to understand the mechanisms and pathways of DNA double helix formation, of protein folding, and of their denaturation and renaturation. A mathematical model of the rates for passing between the various configurational states was previously formulated. Molecular states are specified for each residue as one of a small number of conformations. The effects of an external field in perturbing conformational equilibria have been studied. Local compositional variations provide portions of biological macromolecules which are particularly susceptible to such external effects. In the case of an electric field, these effects can be direct or can be mediated by changes in counterion distributions.

BIBLIOGRAPHIC REFERENCES: Szu, S.C., and Jernigan, R.L.: DNA conformational kinetics. Biopolymers. 18: 83-100, 1979.

261. STUDY OF PROTEIN-NUCLEIC ACID INTERACTIONS

Shifrin, S., Parrott, C. L., Weiss, U., Karpetsky, T., Levy, C. C., Chaiken, I. M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Macromolecular Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this project was to study the nature of the forces through which proteins and nucleic acids recognize one another. The protein examined was pancreatic ribonuclease-A, ribonuclease-S, S-protein, S-peptide and various chemical modifications. The model for the nucleic acid was polyadenylic acid, oligomers of adenylic acid and various derivatives. Interactions were measured by analytical and sucrose gradient ultracentrifugation, as well as by gel chromatography, ultraviolet absorption spectroscopy and fluorescence.

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262. DNA METABOLISM AND DNA BINDING PROTEINS

Esposito, M. S., University of Chicago, Pritzker School of Medicine, Dept. of Medicine, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

This is part of a broader project. A summary of this subproject is not available.

263. ANALYSIS OF DNA MODIFICATION BY MASS SPECTROMETRY

Hattman, S. M., McCloskey, J. A., Nur, U., Angerer, R., Hilf, R., Swinton, D., Smith, D., Crain, P., University of Rochester, School of Arts & Sciences, Dept. of Biology, *260 Crittenden Blvd., River Campus Station, Rochester, New York*, 14627, United States of America

The objective of the proposed research is to develop and use the techniques of gas chromatography-mass spectrometry and high-pressure liquid-chromatography to analyze DNA-modification and its biological role. DNA from various organisms will be examined for the nature/level of methylated (or otherwise modified) bases (e.g., 5-methylcytosine, N6-methyladenine, 1,-3, or -7 methylguanine). Among the systems to be investigated are: (1) the mealybug (to study the connection between DNA modification and facultative heterochromatization); (2) the sea urchin (to study the connection between DNA modification and differentiation), (3) rat mammary tumors (to study the connection between DNA modification and tumor induction, and the role of hormones in these processes).

In addition, we will examine DNAs from unicellular eukaryotes, as well as from bacterial and animal viruses.

264. FLOW CYTOMETRIC DNA ANALYSIS OF HUMAN TUMORS

Vindelov, L. L., Spang Thomsen, M., Engelholm, S. A., Finseninstitutet, Dept. of Internal Medicine, *49 Strandboulevarden, DK2100 Copenhagen, Denmark*

OBJECTIVE: Flow cytometric DNA analysis yields information on ploidy and proliferative characteristics of a cell population. By sequential analysis of treatment-perturbed cell populations, further cell kinetic information can be obtained. The objective of the project is to assess the value of DNA analysis for the subclassification of tumors and for treatment schedule design.

APPROACH: The DNA analysis is performed on fine-needle aspirates of human breast cancer, lung cancer, lymphomas, bladder cancer and cancer of the oral cavity.

PROGRESS: The results show a wide range of the pretreatment characteristics. Clonal heterogeneity has been demonstrated in small cell carcinoma of the lung and in breast cancer. The treatment kinetics of small cell carcinoma of the lung have been described and a treatment protocol based on the cell kinetic changes designed.

265. STATISTICAL ESTIMATION OF DNA DISTRIBUTIONS MEASURED BY FLOW CYTOMETRY

Christensen, I. J., Hartmann, N. R., Keiding, N., Larsen, J. K., Vindelov, L., Finseninstitutet, Finsen Lab., *49 Strandboulevarden, DK2100 Copenhagen, Denmark*

The objective of the project is to develop accurate and fast methods to deconvolute DNA distributions measured by flow cytometry into its G1, S and G2 plus M phase components and/or to determine the ploidy of subpopulations in the sample. It is also desired to correlate the DNA distributions to other parameters measurable by flow cytometry.

The approach to the solution of the problem is based on maximum likelihood estimation using parametric description for the distribution of the S phase.

The method has been tested extensively on experimental and patient data. In the latter case, the method has primarily been used to determine the ploidy of samples taken from small cell carcinoma of the lung and to correlate their DNA distributions to chemotherapeutic treatment.

266. CHARACTERIZATION OF PROSTATIC TUMORS BY FLOW-CYTOFLUOROMETRIC DNA ANALYSIS IN ASPIRATION BIOPSIES

Tribukait, B., Anderson, L., Esposti, P., Ronstrom, L., Karolinska Institutet, Dept. of Medical Radiobiology, *Solnavagen 1, Box 60400, S10401 Stockholm 60, Sweden*

Quantitative DNA analysis has been used to characterize many different types of neoplasms. A correlation has been found between the degree of ploidy, cell differentiation and tumor aggressiveness. We have analyzed aspiration biopsies from various prostatic lesions with a flow-cytofluorometric technique. The results will be correlated with clinical and laboratory data in a follow-up study of untreated and treated cases of carcinoma of the prostate.

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267. FURTHER STUDIES ON THE GENETIC PREDISPOSITION TO CANCER DUE TO DEFECTS OF DNA METABOLISM

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The proposed research is concerned with the genetic predisposition to cancer which may be due to defects of DNA metabolism and is based on the consideration that the identification of subpopulations genetically predisposed to cancer is important for optimal cancer prevention.

It is acknowledged that different factors may contribute to genetic predisposition to cancer but it is felt that defects of DNA replication and repair may not only play an important part in such predisposition but also offer opportunities for a better understanding of carcinogenesis. In particular, a better understanding of the genetics and biochemistry of the defect in xeroderma pigmentosum (XP) seems important not only to understand better the control of nucleotide excision repair in man, but also in order to devise heterozygosity tests. This will be pursued by continuing our studies of the kinetics of complementation in XP and the analysis of nucleotide excision repair in permeabilized cell systems. Comparision of the findings in XP and other photodermatoses without evidence of proneness to skin cancer such as Bloom and Cockayne syndromes will also be made.

268. 5-METHYLCYTOSINE IN DNA - CANCER AND DEVELOPMENT

Ehrlich, M., Gehrke, C. W., Kuo, K. C., Tulane University of Louisiana, School of Medicine, Dept. of Biochemistry, *1430 Tulane Ave., New Orleans, Louisiana*, 70112, United States of America

The only residue in eukaryotic DNA which is modified after polymerization is cytosine which is methylated to give 5-methylcytosine (m5C). Although the average mammalian cell has approximately 4% of its cytosine residues methylated and significant changes in the amount of DNA cytosine methylation have been reported from several different types of cancer cells, extremely little is known about the influence of methylation of DNA cytosine on the physiology of normal or cancerous cells.

We have recently developed two high pressure liquid chromatography HPLC methods for terming the mol % m5C in unlabeled DNA as well as its base composition; these two methods are the most sensitive reported to date. One of these will be used to quantitate m5C levels in DNA from various normal tissues and cell cultures of human, murine, or bovine origin. Also, DNA from many different types of tumor tissue and two types of differentiating cells will be examined. The intragenomic distribution of m5C will be studied by determining the mol% m5C (and base composition) of DNA fractionated according to kinetic reassociation complexity, buoyant density in cesium salt gradients, DNase I-digest dinucleotide composition, pyrimidine isoplith composition, and mitochondrial vs. nuclear origin and resistance to specific restriction endonucleases.

We will also try to isolate from mammalian cells DNA-binding proteins which specifically bind to m5C-rich DNA or regions in DNA. We will use bacteriophage XP-12 DNA as a uniquely m5C-rich DNA to isolate and assay such proteins. It is postulated that such proteins have critical roles to play in controlling transcription or DNA replication and, thereby, in certain phases of differentiation and oncogenic transformation.

BIBLIOGRAPHIC REFERENCES: R. Wang, J. Schedlarski, M. Farber and M. Ehrlich, Two Site-Specific Endonucleases from Xanthomonas oryzae: Characterization and Unusual Properties, Biochim. Biophys. Acta 606: 371-385 (1980). M. Farber and M. Ehrlich, A Phage-Induced Deoxyribonuclease from XP-12 Infected Xanthomonas oryzae, J. Virol. 33: 733-738 (1980).

269. IMPACT OF INTEGRATED FOREIGN DNA VECTORS ON BACILLUS SUBTILIS

Wilson, G. A., University of Rochester, School of Medicine & Dentistry, Dept. of Microbiology, *601 Elmwood Ave., P.O. Box 607, Rochester, New York*, 14642, United States of America

The objective of this project is to elucidate in detail the mechanism of integration of foreign DNA into the genome of Bacillus subtilis and to determine if the presence of foreign DNA alters the physiology of the transformed cell. Recombinant plasmids will be constructed using chromosomal DNA from B. subtilis and related bacteriophages and E. coli vectors to answer the following questions: (1) Where is the site of integration of heterologous DNA in the host chromosome? (2) What is the exact site on the plasmid that is involved in the recombinant event? (3) Can heterologous plasmids be integrated into other plasmids by the same mechanism? (4) Can any gene, once cloned in pMB9, integrate into a host genome harboring pMB9 by virtue of homology between the vector sequences? (5) Are host specific recombination enzymes involved in this integration event? (6) What are the physiological consequences to the cell that has integrated foreign DNA?

We recently observed that chimeric plasmids pCD4 and pCD6 could promote integration of both the foreign DNA and the vector pMB9 into the host chromosome.

This proposal represents an in-depth investigation of the mechanism of that integration event.

270. NUCLEAR DNA STUDY OF GYNECOLOGIC CANCERS

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In this proposed research program, the genetic character of preinvasive and invasive neoplasms of the female genital tract is determined by quantitating the nuclear DNA content by means of Feulgen microspectrophotometry. Ploidy patterns (euploidy, polyploidy and aneuploidy), modal values of stem-cell lines and cell cycle kinetics are analyzed based on their nuclear DNA distribution and correlated with their clinicopathologic features in an attempt to better define the biologic behavior and prognosis of these lesions.

The results of current work indicate that vulvar intraepithelial squamous neoplasms tend to have high ploidy stem cells (DNA modal values greater than triploid), while invasive vulvar squamous carcinomas and carcinomas-in-situ associated with invasive carcinomas have predominantly low ploidy stem cells (less than triploid). Invasive vulvar squamous carcinomas of comparable depth of invasion and size having low ploidy stem cells are more likely to metastasize than those having high ploidy stem cells. Endocervical cancers with high ploidy stem cells have a significantly worse prognosis than those with low ploidy stem cells. In managing the patients with gynecologic cancers, both the nuclear DNA and morphologic changes should be taken into consideration.

271. HYDRODYNAMICS OF CIRCULAR DNA FORMS

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The intracellular replicating form (RF) DNA of coliphage phi X174 has been shown to be heterogeneous with respect to nucleotide sequence. The mature viral form of this DNA, the mature and intracellular RF DNAs of several additional bacteriophages, and plasmid, animal virus, and mammalian mitochondrial DNAs will be examined for such nucleotide sequence heterogeneity. These DNAs, most of which exist as closed circular duplex DNAs or can be so rendered artificially, will be subjected to the introduction of a very limited number of strand breaks per molecule, denatured, renatured, closed with DNA ligase, and the closed circular fractions purified. The susceptibility of these to DNAs to the action of the highly single strand-specific Alteromonas nuclease and their examination by electron microscopy reveals whether lesions, caused by nucleotide sequences in one strand which do not have complementary sequences in the other strand, are present to an appreciable extent. Such lesions serve as substrate sites for the nuclease and appear as single-stranded 'bush' regions in the electron microscope. Control samples are nicked and closed only without denaturation; the Alteromonas nuclease will not cleave the resulting closed duplexes unless lesions are present. These studies will have as their object the determination of whether heretofore unsuspected sequence heterogeneity is present in various small DNA genomes. In other studies, a possible explanation for the apparent increase in superhelical content of negatively super-

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coiled DNAS subjected to the introduction of a limited number of ultraviolet photoproducts will be tested. This explanation, if valid, would demonstrate an important difference between supercoiled and nonsupercoiled DNA at the DNA helix level. The dye-mediated renaturation of denatured closed circular DNA strongly alkaline solutions will also be further investigated, as will the anomalous behavior of phage PM2 closed circular DNA in alkaline solutions. The interaction of DNA topoisomerase I with denatured viral closed circular DNA to produce, among other species, oligomeric forms joined by a novel type of linkage will be further characterized and the nature of the linkage determined.

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272. FUNCTION OF METHYLATED BASES IN NUCLEIC ACIDS

Marinus, M. G., University of Massachusetts, Medical School at Worcester, School of Medicine, Dept. of Pharmacology, *55 Lake Ave. N., Worcester, Massachusetts*, 01605, United States of America

Methylated bases are found in deoxyribonucleic acid (DNA) of bacteria and mammalian cells, but the biological role of such bases is unknown. Escherichia coli and its viruses will be used as model systems to determine the function of 6-methyladenine (6-meA) at the molecular level. The applicant, in collaboration with Dr. N.R. Morris of Rutgers Medical School, has isolated mutant strains (dam mutants) with reduced amounts of 6-meA in DNA. Studies with these dam mutants have shown that they: (1) contain single strand breaks in DNA; (2) are inviable if unable to repair these breaks in DNA; (3) are sensitive to various carcinogenic and mutagenic agents; (4) have an abnormal morphology; and (5) have a high frequency of mutability. These results, together with those of other investigators, suggest that 6-meA is essential for DNA replication, DNA repair and DNA recombination.

The objectives of the present proposal will be (1) to isolate a mutant of E. coli devoid of 6-meA in DNA, (2) to determine which step in phage fd reproduction is blocked in dam repair-deficient mutants, (3) to purify the dam DNA adenine methylase, (4) to purify the hypothetical endonuclease that degrades 6-meA-deficient DNA, (5) to investigate further the hyper-recombination phenotype of dam mutants, and (6) to isolate RNA methylase-deficient mutants.

273. DNA DISTRIBUTION ANALYSIS AND DATA BASE MANAGEMENT

Johnston, D. A., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Biomathematics, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas*, 77030, United States of America

This is part of a broader project. A summary of this subproject is not available.

274. BASE PAIR FLUCTUATIONS AND UNWINDING OF DNA

Opella, S. J., University of Pennsylvania, School of Arts & Sciences, Dept. of Chemistry, *34th & Spruce Sts., Philadelphia, Pennsylvania*, 19104, United States of America

DNA dynamics will be studied using nuclear magnetic resonance spectroscopy. Our results on the phosphodiester backbone of native duplex DNA show that the polymer is flexible, and that the phosphate motion is consistent with the bending motions seen in the hydrodynamic persistence length. This work will be extended to include the sugars and bases of DNA.

DNA binding proteins significantly alter the motions of DNA. The packaging of DNA in filamentous viruses and chromatin will be studied. The unwinding of DNA occurs with temperature, intercalation, or binding to proteins; these influences will be investigated.

These spectroscopic studies of high molecular weight DNA and DNA-protein complexes rely on the use of high resolution solid state NMR techniques. This allows us to study undegraded native samples and to make comparisons among the DNAs of various biological structures.

275. RELATION OF HISTONES TO DNA IN NORMAL AND CANCER CELLS

Kornberg, R. D., Nojima, H., Stanford University, School of Medicine, Dept. of Structural Biology, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

The goal of this research is an understanding of how DNA molecules are coiled in chromosomes and the relationship to gene activity. In addition to work proposed previously, experiments will be done to distinguish between specific and statistical distributions of nucleosomes in the vicinity of coding sequences. The experiments will involve restriction enzyme digestion of chromatin, sucrose gradient sedimentation, and blotting and hybridization with defined DNA sequences.

276. RDNA DISTRIBUTION IN CHROMOSOMES OF NEOPLASTIC CELLS

Henderson, A. S., Megraw Ripley, S., Columbia University, College of Physicians & Surgeons, Dept. of Human Genetics & Development, *630 W. 168th St., New York, New York*, 10032, United States of America

We have demonstrated that certain characteristic morphological features of human D- and G-group chromosomes in cells of patients with proliferative disorders may results from, in part, the presence of rearranged or additional rDNA sites. Using hybridization in situ, rDNA sites have been localized to Q-terminal positions in Dq positive and Dq negative chromosomes, and possibly certain G-group chromosomes. Cytological silver staining indicates that such sites may be transcriptively inactive. It is the focus of the proposed research (a) to determine the extent of rDNA site rearrangement and the possible relationship to chromosome morphology and specific disease state (b) to use several criteria to evaluate whether such sites are transcriptively active or inactive and how this relates to the metabolic state of the neoplastic cell and (c) to analyze possible structural changes in the DNA sequences of the repositioned rDNA complex.

277. ANALYSIS OF THE TRANSCRIPTIONAL PROPERTIES OF THE DNA INSERTION SEQUENCE IS2

Hinton, D. M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Nucleic Acids Research Section, *P.O. Box B, Frederick, Maryland*, 21701, United States of America

The specific aim of this proposal is to characterize the transcriptional properties of the insertion sequence IS2. The proposed studies will focus on IS2 insertions in the galactose operon which turn off gal expression and on IS2 insertions in the int-xis region of phage lambda which turn on int expression. Transcription of the IS2 integrated in these genes will be attempted in vivo and in vitro and the RNA transcripts hybridized to IS2 DNA and characterized by sequencing techniques. These studies will provide information on the potential for transcription and possible translation of the IS2 sequence itself as well as on how insertion sequences affect transcription of adjacent genes.

Evidence exists for the presence of DNA elements analogous to IS insertion sequences in eukaryotes. These studies on bacterial IS elements should provide clues to how such elements function in higher organisms. An understanding of how IS elements transpose and integrate at a variety of sites may also shed light on the mechanism of integration of tumor viruses during transformation.

278. 13C AND OTHER NUCLEIC NMR OF DNA AND DNA INTERACTIONS

Levy, G. C., Rill, R. L., Syracuse University, School of Arts & Sciences, Dept. of Chemistry, *Hall of Languages, Syracuse, New York*, 13210, United States of America

Recent studies in this and other laboratories have shown that 13C, 31P, and 1H nuclear magnetic resonance (nmr) studies of short (100-200 nucleotide pairs-np) double stranded DNA molecules are practical. We propose multi-nuclear (principally 13C), multi-field nmr studies of DNA conformational dynamics and interactions with model carcinogens, toxins and drugs. The overall objective is to relate the conformational dynamics of DNA to normal and aberrant DNA functions in vivo. Specific objectives are (1) to describe the chemical shift environments

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for base and sugar carbons; (2) to elucidate overall and internal DNA motional dynamics using multi-field 13C and 31P nmr data (linewidths, T1's, NOE's) coupled with current theory of relaxation effects in bipolymers, (3) to determine the effects of ionic strength and bulk solvent changes on the conformational dynamics of specific DNA sites, (4) to assess the usefulness of these nmr methods for investigating mechanisms of interaction of small molecules with DNA, and (5) to study in depth those DNA-small molecule interactions judged most amenable to nmr analysis. 13C, and in some cases 31P and 1H nmr spectra will be obtained at high and moderate fields using special optimized probes on the two Florida State University superconducting FT nmr spectrometers. Relatively homogenous length, double stranded DNA subfractions spanning a range from 30 - 200 np will be prepared from native chromatin digested with micrococcal nuclease, followed by gel filtration. Small molecules chosen for study include intercalating agents (proflavine, ethidium, quinacrine, actinomycin D; a steroid diamine (dipyrandium): alkylating agents potentially useful as reporter groups; and heavy metals (Hg ions and Ag ion). 199Hg and other metal nuclide nmr will be used to supplement 13C, 31P, and 1H data in metal binding studies.

279. DNA DYNAMICS AND DRUG INTERCALATION

Sobell, H. M., University of Rochester, School of Medicine & Dentistry, Dept. of Radiation Biology & Biophysics, *601 Elmwood Ave., P.O. Box 607, Rochester, New York*, 14642, United States of America

Crystallographic studies of drug intercalation have led to a series of detailed models to understand drug-DNA (and -RNA) binding. These models all involve conformational changes in the sugar-phosphate backbone characterized by a mixed sugar puckering pattern of the type: C3' endo (3' -5') C2' endo. These conformational changes result from stretching, bending, shearing and unwinding motions of the helix, all tightly coupled.

An important aspect of DNA in solution is its dynamic structure. This reflects the presence of normal mode oscillations in its structure excited by solvent collisions at thermal energies. A key intermediate in the process of drug intercalation into DNA is the 'kink', a conformational change in the sugar-phosphate backbone due to wave (or acoustic) phonon-propagation in the polymer. Once formed, the kink can then be likened to a flexible hinge in DNA -- this hinge can straighten and base-pairs separate to provide space for drug intercalation.

This research proposal describes cyrstallographic studies in progress that are designed to deepen our understanding of drug intercalation into DNA. Attempts will be made to crystallize the kink by binding small oligonucleotides to the steroidal diamine, irehdiamine A (a molecule, we postulate, binds to the kink in DNA). Other studies include understanding the mechanism of action of such anticancer agents as daunorubicin, a DNA intercalating agent. We are also concerned with understanding the mechanism of action of frameshift mutagens, such as 9-aminoacridine, acridine orange and proflavine. Our crytallographic studies have provided new information that is probably relevant to understanding their mutagenic properties; we will describe these in this proposal.

280. ASSEMBLY AND TRANSCRIPTION OF ACTIVE CHROMATIN

Weisbrod, S., Medical Research Council, Lab. of Molecular Biology, *15 Chaucer Rd., Cambridge CB2 2BF, England, United Kingdom*

The aim of this proposal is to try to elucidate the mechanism whereby a naked piece of DNA is assembled into actively transcribing chromatin. Active genes are packaged into nucleosomes and have a structure different from that of the bulk of the nucleoprotein. Recent studies have shown that two nonhistone nuclear proteins, termed HMG 14 and 17, are associated with genes which are active or have been active in the lifetime of the cell. Using techniques developed in the laboratories of John Gurdon and Ronald Laskey I would like to investigate how and when HMG 14 and 17 are associated with or assembled into the nucleosome, and how they affect the fidelity and mechanism of transcription. The hope is that we can use the information gained by these two lines of experiments to investigate the more profound question of timing, that is, how specific genes are recognized and turned on at certain stages of development.

281. CONFORMATIONAL STATES OF CHROMATIN USING SITE-SPECIFIC INTERCALATING DRUGS AS PROBES

Schmitz, K. S., University of Missouri, Kansas City Campus, School of Arts & Sciences, Dept. of Chemistry, *5100 Rockhill Rd., Kansas City, Missouri*, 64110, United States of America

The objective of the proposed research is to determine the conformational states of chromatin as reflected in the accessibility of specific sites. Chromatin has a repeat unit (nucleosome) structure which is composed of two copies each of the histones H2a, H2b, H3, and H4 and approximately 200 base pairs of DNA. Only 140 base pairs of DNA are in tight contact with the histone core (core particle) while the core particle substructures are linked by the remaining DNA in the nucleosome (linker DNA). Another class of histones, the lysine rich histones, are assumed to be associated with the linker DNA. The conformational states of the mono- and poly- nucleosomes will be detected in the proposed study by using site-specific probes proflavine (A-T specific, major groove), ethidium bromide (not base pair specific, major groove) and actinomycin D (G-C specific, minor groove). Binding sites on the core particle and linker DNA will be characterized by binding isotherms (intrinsic binding constant, remote-neighbor interaction parameter, and number of excluded sites) determined by difference absorption spectroscopy and difference fluorescence spectroscopy. The effect of ligand binding on the hydrodynamic conformation of the nucleosome(s) will be determined by quasielastic light scattering, electrophoretic light scattering, and sedimentation velocity techniques. The goals of the proposed project are to: 1) characterize the conformational states of the core particles and mononucleosomes; 2) characterize ligand-oligonucleosome complexes; and 3) determine the location of lysine rich histone binding sites.

282. POLYMORPHISM IN HUMAN DNA

Barker, D. F., University of Massachusetts, Medical School at Worcester, School of Medicine, Dept. of Microbiology, *55 Lake Ave. N., Worcester, Massachusetts*, 01605, United States of America

The goal of our projected research is to develop the use of restriction fragment length polymorphisms (RFLPs) as a tool for human genetic mapping. Studies of specific genetic regions in several organisms including yeast, chickens and humans have revealed that, although the DNA of different individuals is largely identical in nucleotide sequence, occasional differences are observed which may be detected as changes in the pattern of cleavage of the DNA by sequence-specific endonucleases. These differences, called RFLPs, constitute a newly recognized source of genetic polymorphism. Because the means for determining the allelic state of any individual are reasonably efficient, it is possible to make use of these structural alterations as genetic markers. By developing a set of probes able to detect polymorphic loci dispersed throughout the human genome, we hope to establish a means for detecting genetic linkage between loci determining important human traits, such as susceptibility to certain diseases, and specific RFLPs. Detection of such linkage will permit detection of disease locus carriers, antenatal diagnosis and the analysis of environmental and genetic factors influencing the expression of these traits in individuals.

283. CARCINOGENESIS AND DNA CONFORMATIONS

Rich, A., Massachusetts Inst. of Technology, School of Science, Dept. of Biology, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

II. CONTROL OF GENE EXPRESSION AND DNA TRANSCRIPTION

284. A MONOCLONAL ANTIBODY LIBRARY TO DROSOPHILA NUCLEAR PROTEINS (APPLICATIONS TO GENE EXPRESSION AND DNA REPLICATION)

Sedat, J. W., Alberts, B., McCarthy, B., Kuo, C., Blumenthal, A., University of California, San Francisco Campus, School of

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Medicine, Dept. of Biochemistry, *551 Parnassus Ave., San Francisco, California,* 94122, United States of America

The objective of this proposal is to construct a 'library' of monoclonal antibodies to Drosophila nuclear proteins. New methods will be used to prepare the nuclear protein antigens. The monospecific antibodies produced against these proteins will be characterized and classified by the biochemical properties of the protein antigens bound by the antibodies and by the in situ localization of these antigens in Drosophila polytene chromosomes and in other Drosophila tissues. It is proposed that the cell clones producing these well-characterized antibodies will be a national resource.

Initially, four uses of this library are proposed using Drosophila. First, nuclear protein antigens will be visualized on spread chromatin that is active in transcription and replication. Electron microscope methods, using monoclonal antibodies will be developed. Second, monoclonal antibodies will be used to identify possible regulatory proteins that bind specifically to the genes that are activated by heat-shock. Third, proteins possibly involved in DNA replication will be identified by monoclonal antibody methods. Indirect and direct functional assays will be developed to define the roles of these proteins in DNA replication. Fourth, non-histone nuclear proteins ttat are possibly involved in determination steps during early embryonic development will be identified and studied using the proposed antibody methods.

This research will provide new tools generally applicable to the problems of gene regulation and replication in eukaryotic cells and will provide a biochemical approach to the genetic manipulation of these processes.

285. CHROMATIN STRUCTURE AND GENE EXPRESSION IN DROSOPHILA

Elgin, S. C., Harvard University, Cambridge Campus, School of Arts & Sciences, Dept. of Biochemistry & Molecular Biology, *Cambridge Station, Cambridge, Massachusetts,* 02138, United States of America

The DNA of eukaryotes is associated with histones and nonhistone chromosomal proteins (NHC proteins) in a complex structure referred to as chromatin. We wish to determine in detail the differences in chromatin structure related to gene activity and to relate the map of these differences to other features of genome structure. In particular, we plan to test the hypothesis that certain chromatin structures which can be detected in terms of nuclease sensitivity are necessary for gene activation and transcription. Drosophila is the organism of choice for our work because of the facility with which biochemical, genetic, and cytological techniques can all be utilized. Using DNase I and micrococcal nuclease as probes, we plan to map the chromatin structure (position of DNase I hypersensitive sites, position of nucleosome sites, boundaries of regions of perturbation on gene activation) for several different types of loci, including a generally inducible locus (heat shock locus 67B), a developmentally regulated locus (vitellogenin), and a locus expressed at a low constant level (encoding a ribosomal protein). Finally, we wish to characterize a developmentally regulated locus at several stages leading to gene activation. To this end we are proceeding to clone a gene expressed late in tissue culture cells in response to ecdysterone. A similar analysis will be carried out on this gene. Ultimately we hope to establish this gene on a minichromosome, present in the cell in multiple copies, to proceed with an analysis of the chromosomal proteins involved in these structural transitions.

286. THE REGULATION OF GENE EXPRESSION DURING DEVELOPMENT IN DROSOPHILA MELANOGASTER

Kingsman, S. M., University of Oxford, Dept. of Biochemistry, *S. Parks Rd., Oxford OX2 6HE, England, United Kingdom*

OBJECTIVE: To generate a series of cloned DNA sequences from the genome of Drosophila melanogaster contining genes which are expressed differentially during development. These cloned sequences will be used as hybridization probes to follow the synthesis and degradation of corresponding messenger RNA (mRNA) species, to analyze the structure of these mRNAs and to determine possible regulatory mechanisms governing differential gene expression. Particular emphasis will be placed on the role of beta-ecdysone in regulating coordinate gene expression during larval metamorphosis.

APPROACH: Differential plaque hybridization is being used to screen a collection of E.Co.RI fragments of the Drosophila genome inserted into the bacteriophage lambda vector for developmentally regulated genes. Probes and 32P-labelled complementary DNA (c-DNA) copies of poly-A RNA isolated from organisms at different developmental stages, i.e., embryos, larvae, pupae and adults, from imaginal disc organ cultures and various tissue culture cell lines before and after treatment with beta-ecdysone and from larvae before and after in situ beta-ecdysone action. The cloned sequences will be used in filter hybridizations to quantitate the changes in corresponding nuclear and cytoplasmic RNA levels during development. Transcripts will be mapped by standard hybridization and gel electrophoresis techniques and 5' regions and flanking regions sequenced to identify possible regulatory sequences. In particular, coordinately regulated genes will be compared to identify any common sequences. Cloned sequences will be located in the Drosophila genome by in situ hybridization to polytene chromosome to determine if any known loci have been cloned, if there is any clustering or if there is any repetition of particular sequences.

287. ANALYSIS OF STEPS CONTROLLING GENE EXPRESSION IN DROSOPHILA

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We are investigating the contributions of transcription, processing and turnover to the expression of specific genes in Drosophila cultured cells and embryos. We have evaluated these parameters in detail for the RNA transcribed from the copia sequence, a mobile DNA sequence in the Drosophila genome. In order to understand the role which the copia sequences and mobile DNA elements in general play in the cell, we are now investigating the translation product of copia RNA. In order to compare the control of expression of the copia sequence, which is expressed continuously in the cultured cells, with the control of expression of genes which are induced, we are currently measuring the parameters of synthesis, processing and turnover for two genes which are induced to a high level of expression by the stimulus of heat shock. Finally, we have demonstrated that the level of histone gene expression in Drosophila embryos is controlled both by changing rates of synthesis and by changing rates of turnover. We are currently investigating different mechanisms by which the control of histone mRNA turnover might be controlled.

BIBLIOGRAPHIC REFERENCES: Pardue, M.L., Scott, M.P., Storti, R.V. and Lengyel, J.A. (1980) The heat shock response: a model system for the study of gene regulation in Drosophila. In: Development and Behavior of Drosophila melanogaster (eds., O. Siddiqi, L. Hall and P. Babu), Plenum Press, Basic Life Science Series, New York, in press. Stanfield, S.W. and Lengyel, J.A. (1980) Small circular DNA of Drosophila melanogaster: homologous transcripts in nucleus and cytoplasm. Biochemistry, in press.

288. ORGANIZATION AND EXPRESSION OF GENETIC INFORMATION IN LOWER EUKARYOTES

Kramer, R. A., Andersen, N. J., Thill, G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Nuclear Acid Enzymology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

This project employs recombinant DNA techniques to isolate individual genes of yeast. These clones are then used in studies of the organization and expression of these genes at the molecular level.

BIBLIOGRAPHIC REFERENCES: R.A. Kramer and J. Smallwood (1978). Detection of yeast ribosomal RNA sequences in E. coli infected with hybrid bacteriophage. Nucleic Acids Res. 5, 4725-4736.

289. GENE EXPRESSION IN XENOPUS LAEVIS

Dworkin, M. B., Dawid, I. B., Wood, B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Developmental Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

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We have previously constructed a cDNA library derived from RNA of Xenopus laevis embryos. This library has been used to compare the abundance of about 200 RNA species in different developmental stages and cellular fractions of X. laevis. Developmental profiles for these RNA species were obtained.

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290. MOLECULAR BASIS FOR HISTONE GENE EXPRESSION DURING ANIMAL EMBRYOGENESIS

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The research proposal is directed at determining the molecular basis of histone gene expression. In the sea urchin S. purpuratus, a distinct shift in histone mRNA synthesis occurs at the 11th-12th hour of embryogenesis. This shift has been shown to result from the activation of a separate class of 'late' histone genes. I will clone large fragments (20 kilo bases) of S. purpuratus histone genes into bacteriophage lambda using partial restriction enzyme digests, synthetic Eco R1 linkers and in vitro packaging of lambda. Each recombinant phage containing histone genes will be classified into 'early' and 'late' types using restriction enzyme digests, and a comparison of these patterns with that of a cloned 'early' histone repeating unit, which is already available, will be made. The majority of the recombinant clones will thus be eliminated and the remaining clones examined for 'late' histone genes. To isolate a 'late' histone gene clone, I will use a 'late' histone mRNA probe to hybridize to each potential 'late' histone gene clone and determine RNase sensitivity and thermal stability of the hybrid molecules. I will examine both the long-range organization and fine structure of the 'late' histone clone and compare these with the known structure of the 'early' histone genes. The most likely regions involved in gene control are those immediately preceding the beginning of the coding region. I intend to sequence the area adjacent to the H4 gene in the 'late' histone clone, using rapid ladder sequencing techniques, and compare the sequence with the published sequence of the corresponding region in the 'early' H4 gene. The 'early' and 'late' genes diverged over 175 million years ago, and controlling regions, which are expected to be highly conserved sequences, may be detectable. This will support future research in Dr. Grunstein's laboratory, in which the corresponding regions in both the 'early' and 'late' H4 genes of L. pictus will be examined.

291. HISTONE GENE EXPRESSION IN THE DEVELOPING SEA URCHIN EMBRYO

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Development and differentiation of the embryo must in large part be controlled by gene activity prior to and during embryogenesis. To study the process of activation and deactivation it is necessary to use a gene system that is modulated during development and is amenable to biochemical analysis. Histone genes are activated during oogenesis, and then after fertilization, in a unique manner.

At the mid-blastula stage (12 hours of development) there is an abrupt change in the histone mRNA synthesis pattern. Over a period of several hours the early histone mRNAs are gradually replaced on the polysomes by a new class of histone mRNAs. This new class, the late histone mRNAs, differs in both size and sequence from the early mRNAs and are coded for by a new (late) gene class.

Experiments in this proposal, utilizing DNA technology, will examine in detail the sequence structure of maternal, early and late histone mRNAs and the organization of the genes or gene clusters coding for these mRNAs. Furthermore, experiments are designed which will analyze in vivo and in vitro the precursor transcripts of histone genes utilizing sea urchin nucleii as the first step in setting up a means for studying histone gene control.

292. GENE EXPRESSION IN THE CYTOCHROME C GENES OF YEAST

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The aim of this proposal is to study the relationship of gene expression to DNA sequence in the cytochrome c genes of yeast. Regulatory mutants of the iso-2-cytochrome c gene will be isolated, both wild type and mutant genes will be cloned and sequenced with emphasis on the regulatory region flanking the structural gene. Cloned cytochrome c genes will then be used to transform mutant yeast strains and the regulation of these inserted genes will be investigated. The isolated and sequenced genes will be the basis for future studies on cytochrome c mRNA metabolism and the possible isolation of regulatory proteins.

293. EXPRESSION OF SEA URCHIN HISTONE GENES IN E. COLI

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The basic aim of my proposal is to design a methodology for obtaining expression (translation) of eukaryote genes in E. coli, and then apply bacterial molecular biological tools to study a eukaryote problem. The genes I propose to study are the histone genes H3 and H4 of the sea urchin P. miliaris. I have chosen this system for several reasons.

The histone locus of P. miliaris has already been cloned in E. coli by recombinant DNA techniques, and a restriction enzyme map of the region exists. Thus, it is possible to apply to these genes new cloning techniques designed to obtain efficient expression of the gene which is cloned. Success in gaining high level expression of a histone gene in E. coli itself would be a very significant result.

In vitro reconstitution experiments with histones H3 and H4 and DNA suggest that expression of H3 and H4 together in E. coli would alter the structure of host DNA. In such a cell, it would be possible to isolate histone mutants defective in function. A mutant histone protein could be purified readily, since it would be expressed at a high level. The purified mutant histone could be studied in a reconstitution experiment with DNA, as this system is well characterized. Thus, a detailed mutational analysis can be performed to probe protein-protein and protein-DNA interactions in chromatin.

294. REGULATION OF TRYPANOSOME GENE EXPRESSION

Marcu, K. B., State University of New York, Stony Brook Campus, School of Arts & Sciences, Dept. of Biochemistry, *Stony Brook, New York*, 11790, United States of America

African trypanosomes have evolved a highly complex mechanism for camouflaging themselves from the immune defenses of their mammalian hosts. This phenomena is manifested through their seemingly endless repertoire of antigenically distinct but homogeneous surface glycoproteins (Variant Specific Surface antigens, VSSAs or VSGs). The potential for VSG expression fluctuates during the trypanosome life cycle which consists of several morphologically distinct differentiated forms.

We have prepared a cloned complementary DNA (cDNA) sequence for one specific VSSA from T. brucei. Southern blot hybridization analyses of restriction enzyme digestions performed with our cloned VSSA cDNA probe on the genomes of two T. brucei clones expressing different VSSA genes have demonstrated that their active and inactive gene states are contextually different. We would like to analyze this phenomenon in detail to determine whether somatic recombination or gene mobility are in part responsible for VSSA gene expression and potentially the VSSA diversity.

Recombinant DNA techniques will be employed to: (a) prepare cDNA clones for other temporally related VSSA mRNAs, (b) assess the nucleotide sequence homologies of antigenically distinct as well as isotypically related VSSA, (c) determine the overall organization of VSSA genes, (d) analyze the extent of the VSSA gene repertoire, (e) ascertain the potential role of DNA recombination in VSSA repertoire expansion, (f)

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determine whether RNA processing and splicing of VSSA nuclear transcripts are in part responsible for the regulation of VSSA gene expression, (g) to determine whether the expression of other, possibly less abundant trypanosome gene products are trypanosome class specific and whether these coordinate or non-coordinate expression with VSG's.

295. REGULATION OF THE ALPHA 2 COLLAGEN GENE DURING DIFFERENTIATON AND REGULATION OF GENE EXPRESSION IN BACTERIA

de Crombrugghe, B., Pastan, I., Adams, S., Sobel, M., Avvedimento, E., Ohkubo, H., Vogeli, G., Mudryj, M., Yamada, Y., Busby, S. J., Aiba, H., Maizel, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Gene Regulation Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

To study the control of a differentiation specific gene, we have isolated the chick alpha 2 collagen gene. The exons of the chick alpha 2 collagen gene are small and of similar size. This suggests that this gene arose by amplification of a single coding unit of unique size (54 b.p.)

Removal of intervening sequences occurs often in more than one step by splicing at sites within introns. These internal splice sites exhibit the same sequence complementarities with a small nuclear RNA, known as U1 RNA, as the end of introns.

The levels of alpha 2 collagen mRNA in the cytoplasm and those of collagen RNA precursors in the nucleus are severely reduced in Rous sarcoma virus transformed cells, strongly suggesting that control of this gene occurs at the level of stranscription.

A model has been developed for the interaction of the E. coli cyclic AMP receptor subunits with their recognition sites in the gal, lac and ara promoters.

BIBLIOGRAPHIC REFERENCES: Showalter, A.M., Pesciotta, D.M., Eikenberry, E.F., Yamamoto, T., Pastan, I., de Crombrugghe, B., Fietzek, P.P., and Olsen, B.R. Nucleotide sequence of a collagen cDNA-fragment coding for the carboxyl end of pro alpha 1 (I)-chains. FEBS Letters. 111: 61-65, 1980. Court, D., de Crombrugghe, B., Adhya, S., and Gottesman, M. Bacteriophage lambda hin function: II. Enhanced stability of lambda messenger RNA. J. Molecular Biology, in press, 1980.

296. CONTROL OF GENE EXPRESSION IN THE E. COLI K12 GAL OPERON AND BACTERIOPHAGE LAMBDA

Adhya, S. L., Irani, M., Ward, D., Gottesman, M. E., Gulletta, E., Garges, S., Reyes, O., Merril, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biochemical Genetics Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

We have continued our study of the expression of the gal operon E. coli; either from the P1 and P2 promoters of the gal operon or from the PL or PR promoters of a neighboring lambda prophage. The expression of operon is strongly influenced by the promoter which is chosen for its transcription. This control is exerted at both transcriptional and translational levels.

We are also continuing our study on the nature of transcription termination in E. coli, and of the mechanism of action of the bacteriophage lambda anti-termination-function, the product of the N-gene.

We have also expanded our study on the biochemical and physical characterization of the gene that controls the transcription termination factor Rho.

BIBLIOGRAPHIC REFERENCES: Greenblatt, J., Li, J., Adhya, S., Friedman, D., Baron, L., Redfield, B., Kung, H., and Weissbach, H. Evidence that the L factor required for DNA dependent in vitro synthesis of beta-galactosidase is the E. coli nusA gene protein. Proc. Natl. Acad. Sci. USA, in press, 1980. Gottesman, M., Adhya, S., and Das, A. Transcription antitermination by lambda N-gene product. J. Mol., Biol., in press, 1980.

297. EXAMINATION OF NUCLEIC ACID STRUCTURAL AND FUNCTIONAL RELATIONSHIPS INVOLVED IN REGULATING CELLULAR AND VIRAL GENE EXPRESSION

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We have been investigating nucleic acid structure involved in the control of transcription initiation & termination as well as translational expression of various RNA transcription products. Using recombinant DNA techniques we have been able to examine the relationship between regulatory function and DNA sequence in a variety of bacterial and viral operon control regions. The characterization of mutants which affect both transcriptional and translational expression in these regions has afforded information on the precise molecular events which lead to control element function.

We have used a variety of defined prokaryotic mRNAs to examine the requirements for translation in a eukaryotic cell-free system. Our results indicate that analogous to many eukaryotic mRNAs, efficient translation of the prokaryotic mRNAs is obtained only upon modification of their 5' termini with a 7-CH3-guanosine 'cap' structure.

In collaboration, we have also been characterizing a 172 bp DNA segment which is repeated millions of times in the genome of the African Green Monkey.

BIBLIOGRAPHIC REFERENCES: McKenney, K., Shimatake, H., Court, D., and Rosenberg, M.: A system to study transcription regulatory signals in single and multiple copy. Fed. Proc. 39: 2203, 1980. Wulff, D.L., Beher, M., Izumi, S., Beck, J., Mahoney, M., Shimatake, H., Brady, C., Court, D. and Rosenberg, M.: Structure and function of the cY control region of bacteriophage lambda. J. Mol. Biol. 138: 209-230, 1980.

298. CONTROL OF HOST GENE EXPRESSION BY DNA TUMOR VIRUSES

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This research project is directed at understanding the effect of DNA tumor virus infection on host gene expression. Studies are focused primarily on investigating the synthesis of one particular virus induced cellular enzyme, dihydrofolate reductase (DHFR). For these studies we have used methotrexate (MTX)-resistant mouse cells in which DHFR is a major gene product, accounting for as much as twenty percent of the soluble protein in some cell lines. In these cell lines DHFR synthesis is subject to control by several important, and possibly related parameters including polyoma infection, growth stimulation, and cyclic AMP.

299. REGULATION OF GENE EXPRESSION IN DNA TUMOR VIRUSES

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Transcription of adenovirus 2 RNA sequences specified by the early gene region responsible for cell transformation will be studied in both lytically infected and transformed cells. Distinct viral nuclear RNA species will be identified and precisely mapped within the viral genome by hybridization to various restriction endonuclease fragments of viral DNA. Pulse-chase experiments will be done to study the metabolism of these RNAs, and to establish a precursor-product relationship between nuclear viral RNA and cytoplasmic mRNA. A system using isolated nuclei from early-infected cells will be developed to study the metabolism of nuclear viral RNAs; RNAs synthesized in isolated nuclei will be compared with those made in intact cells. The effects of protein synthesis inhibitors (cycloheximide) and of temperature-sensitive mutations in the viral genome on the transcription of these RNA sequences will be investigated in infected and transformed cells.

300. CONTROL OF CELL DIVISION IN LEUKEMIA CELLS

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Previous studies have shown that the cell surface expression of thymus leukemia antigen (TL) on ASL-1w leukemia cells varies with the progression of the cells through the growth cycle. Expression of TL is maximal in S phase and its quantita-

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tive expression varies directly with DNA synthesis. In the present study the effect of anti-TL serum on the growth of ASL-1w cells was examined. The antiserum, tested in the absence of complement, affected the growth of these cells in biphasic manner. When the antiserum concentration was 0.1% or greater, there was a rapid decline in the rate of DNA synthesis and, after 5-7 hours, cell death occurred. When the antiserum concentration was 0.057% or less, the decline in the rate of synthesis of DNA did not become apparent until 5-6 hours after treatment. Under these conditions there was approximately a 20% increase in cell number after 24 hours of culture. The hypothesis that treatment of ASL-1w cells with the lesser concentration of anti-TL serum blocks the cells in G-2 phase of the cell cycle is discussed. The objective of this proposal is to test this hypothesis. Relevant experiments which will be carried out are: 1) Biosynthesis of TL and relationship of cell cycle to regulation. 2) Use of various cell cycle specific drugs to determine their effect on the regulation of biosynthesis of TL. 3) Use of synchronous cell cultures to study the relationship between TL and regulation of cell division.

BIBLIOGRAPHIC REFERENCES: Wolcott, M., Pickering, J.W. and Williams, J.L.: Inhibition of Growth of ASL-lw Murine Leukemia cells by anti-thymus leukemia antigen (TL) serum in the absence of complement. J. Immunology 123 1985-1988 (1979). Pruett, S.B. and Wolcott, M.: Quantitation of proteins in the femtomole range by hemagglutination using an electronic particle counter. J. Immunological Methods 35 129-136 (1980).

301. REGULATION OF ALPHA-1-FETOPROTEIN GENE EXPRESSION

Belanger, L., Anderson, A., Dauphinais, C., Hotel Dieu de Quebec, Molecular Oncology Laboratory, *11 Cote du Palais, Quebec City, Quebec*, G1R 2J6, Canada

OBJECTIVE: To elucidate the mechanisms which control the expression of alpha-1-fetoprotein (AFP) during development and malignant transformation of rat liver.

APPROACH: The identification and mode of action of AFP gene effectors is approached prinicipally through nonhistone protein characterization, nuclear and chromatin transcription in vitro, chromatin reconstitutions, and cell hybridizations. Comparative studies are made with the albumin gene. cDNA probes serve to measure mRNA sequences. Particular attention is devoted to glucocorticoid suppression of AFP in newborn liver.

PROGRESS: AFP and albumin mRNAs have been purified and cDNA probes have been synthesized. Cloning of the cDNAs and amplification of genomic sequences are in progress. Nuclear and chromatin transcription using the mercuri-nucleotide methodology is currently characterized. During normal liver ontogenesis and in malignant states, the AFP gene function appears mostly regulated through transcriptional mechanisms. Dexamethasone suppresses AFP in newborn liver with a high degree of selectivity and very probably at transcriptional level. Normal and malignant liver cells express the AFP gene differently in hybrids. Identification of the AFP chromosome through cell fusion is in progress.

302. ASPECTS OF CONTROL IN MAMMALIAN GENE EXPRESSION

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We will use cloned genomic fragments from the 1000-kb region of DNA amplified in PALA-resistant hamster cells to investigate the details of the amplification process, especially important structural features of the amplified unit, whether or not each cloned region is amplified to the same extent in independent events, whether or not transcriptional units other than the CAD gene are present within the amplified region and, if so, whether or not they are expressed in proportion to gene copy number. We also plan to look at cells early in the process of gene amplification in an attempt to characterize early structures and events in the process.

We will also continue to characterize SV40 transcriptional complexes obtained late in infection, and to investigate the possibility that interferon blocks an uncoating event early in SV40 infections.

BIBLIOGRAPHIC REFERENCES: Reiser, J., J. Renart, L.V. Crawford and G.R. Stark (1980) Specific Association of Simian Virus 40 Tumor Antigen with Simian Virus 40 Chromatin, J. Virol. 33, 78-87. Llopis, R. and G.R. Stark (1981) A Deletion within the Genes for the Simian Virus 40 Structural Proteins VP2 and VP3 Leads to Formation of Abnormal Transcriptional Complexes, J. Virol., in press.

303. TRANSLATIONAL CONTROLS OF GENE EXPRESSION IN MESSENGER RNAS

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The research proposed in this application is designed to evaluate the contributions made by messenger RNA ribosomal RNA interaction, initiator codon sequence, and mRNA secondary structure in determining the specificity of ribosome recognition and the overall efficiency of a translation initiation site. Three sets of initiation sites currently under study by the principal investigator have been selected for further investigation. (1) The initiator for the wild type lac repressor protein of E. coli and internal reinitiation sites activated by nonsense mutations early in the I gene which encodes the repressor will be examined. Experiments are proposed to analyze functional determinants in these initiators and to determine which mRNA sequence and structural features underlie the apparent specificity of reinitiation site selection. Site-directed in vitro mutagenesis techniques will be applied to obtain I gene mutants which exhibit altered translation initiation. Such mutants will be scored for their effects on in vivo gene expression, and will be analyzed using the combined techniques of mRNA and DNA sequencing, ribosome binding, and N-terminal amino acid sequencing. Analogous studies will be initiated on (2) the bacteriophage gamma N gene messenger RNA, and (3) the overlapping set of polycistronic mRNA which appear as the major primary and processed transcripts in E. coli infected with the filamentous single-stranded DNA bacteriophage f1. The genetic and nucleotide sequence information available for each of these systems make them ideal for further study, and should lead to substantial contributions by these investigations to our understanding of the molecular interactions which underlie and regulate the polypeptide chain initiation step during gene expression.

304. STRUCTURE OF THE GENE FOR G3PDH IN MAMMALIAN CELLS AND REGULATION OF ITS EXPRESSION IN NORMAL AND TRANSFORMED CELLS (ABBREV)

Jeanteur, P., Blanchard, J. M., Cathala, G., Milhaud, P., Piechaczyk, M., Universite de Montpellier II - Univ. Scis et Tech. du Lang., Laboratoire de Biologie Moleculaire, *Pl. Eugene Bataillon, 34060 Montpellier, France*

The summary for this project is under entry no. 8.

305. INSULIN GENE EXPRESSION IN NEOPLASIA

Duguid, J. R., University of Chicago, Pritzker School of Medicine, Dept. of Biochemistry, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

We have detected four DNA fragments containing sequences complementary to insulin mRNA in endonuclease EcoR1 digests of rat spleen DNA, using the Southern technique with 125I-labeled insulin mRNA as a probe. Each of these fragments has been purified approximately 200-fold by actinomycin D-cesium chloride centrifugation, RPC-5 chromatography, and agarose gel electrophoresis. We plan to ligate these DNA preparations into the EK-2 vector lambda-gt WES, transfect E. coli strain LE-392 with the recombinant molecules, select clones containing insulin-specific DNA by plaque hybridization with 125I-insulin mRNA, and amplify the positive recombinants in accordance with NIH guidelines. The DNA fragments thus obtained will be characterized by sequence analysis of regions complementary to our probe. The characterized DNA fragments thus obtained will be used to purify nuclear RNA species containing insulin-specific sequences from a rat insulinoma. We have detected two high molecular weight RNA species which by hybridization analysis appear to contain sequences present in insulin mRNA. We hope to study the possi-

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ble conversion of these larger molecules to cytoplasmic messenger RNA by pulse-chase techniques and by physically mapping sequence homologies between the nuclear RNAs and cloned insulin-specific DNA using the electron microscope.

306. INDUCTION AND REPRESSION OF GENE EXPRESSION BY AMINO ACID ANALOGUES

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The proline analogue azetidine-2-carboxylic acid (AzC) induces drastic alterations in the pattern of gene expression in HeLa cell cultures. Synthesis of the majority of normal cell proteins is depressed whereas the production of a characteristic set of about 5 polypeptides is induced. Upon prolonged exposure, some of the AzC-induced proteins become major constituents of the cell. Various other cell types exhibit similar responses to alternative stimuli of diverse nature. We propose: 1) To elucidate the nature and properties of the AzC-induced proteins, to determine their relationships to one another, to normal cell proteins and to the proteins induced by other stimuli. In addition, antibodies directed against the induced proteins will be prepared. 2) To investigate the alterations in RNA metabolism which accompany induction and deinduction to discover the fate of preexisting mRNAs and to characterize the induced mRNAs. 3) To prepare cloned copies of the mRNAs coding for the induced proteins and use these to probe the structure of their genes and the mechanism of induction. 4) To analyse the induction mechanism using selective inhibitors of macromolecular synthesis, specific probes and cell-free protein synthesizing systems. 5) To further investigate the response to AzC using cell mutants which are resistant to the inducing effects of AzC.

307. IMMUNOGLOBULIN GENE EXPRESSION IN MYELOMA VARIANTS

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The goal of this research is a greater understanding of the regulation of the expression of immunoglobulin genes in mouse myeloma cells. We propose to analyze the regulation of immunoglobulin production by studying variants of the myeloma cells which produce deleted heavy chains, proteins and variants which have undergone a switch of their heavy chain constant region genes. We propose to analyze the genomic DNA, hnRNA and mRNA of the variants to precisely define the alterations observed. The hnRNA and mRNA of the deletion mutants will be studied by analyzing their migration on gels and hybridization to constant region domain enriched probes on gels. The DNA of the mutants will be purified, cloned in phage vectors and restriction enzyme maps compared to wild type. Sequences of DNA at deletions and gene switches will be compared to wild type (w.t.).

308. CHROMOSOMAL MANIFESTATIONS OF GENE EXPRESSION

Melera, P. W., Biedler, J. L., Peterson, R. H., Sloan Kettering Inst. for Cancer Research, Laboratory of RNA Synthesis & Regulation, *410 E. 68th St., New York, New York,* 10021, United States of America

The proposed work seeks to (1) provide biochemical evidence, and a genomic localization, for DNA sequence amplification in antifolate-resistant Chinese hamster cells which overproduce the enzyme dihydrofolate reductase, DHFR; (2) demonstrate identity between the putative DNA amplification event and HSRs (homogeneously staining regions) revealed by G-banding of metaphase chromosomes; and (3) determine the mechanism of overproduction of DHFR in these cells. With the use of combined cytological, molecular biological, and recombinant DNA techniques, which will include sister chromatid exchange analysis, mRNA purification, sequencing, cDNA synthesis via reverse transcription, in vitro labeling of DNA using 'nick translation,' Cot and Rot sequence complexity analysis, and cytological hybridization in situ, we will also attempt to provide evidence for structural gene amplification as a previously unrecognized mode of gene regulation in mammalian cells.

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309. EXPRESSION OF TRANSFERRED EUKARYOTIC GENES IN MAMMALIAN CELLS

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The overall objective of this proposal is to study the structural organization and expression of the herpes simplex virus thymidine kinase (tk) gene in mammalian cells transformed by restriction fragments containing the tk gene. Experiments will be focused on the transcriptional events of tk gene expression in an effort to (a) identify thymidine kinase specific mRNAs and map their coding region; (b) investigate possible intervening sequences in tk gene organization; (c) structurally perturb the tk gene with specific deletions and changes by in vitro mutagenesis and use them to probe the processes of transcription and processing; and (d) use the tk gene as a vector for introducing other eukaryotic genes (e.g., globin) and studying their expression as well.

310. MODULATION OF GENE EXPRESSION IN CELL CULTURE

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OBJECTIVES: 1) The ectopic production of polypeptide hormones by non-endocrine cells provides a model for analyzing the mechanisms responsible for gene expression. 2) The elucidation of simple chemical compounds capable of activating the expression of specific genes provides an approach to analyzing gene regulation. 3) Understanding of molecular events involved in gene activation provide insights into the control of specific protein synthesis with implications for normal differentiation and perhaps for the regression of normal function observed in neoplasia.

PLAN: To characterize the alterations and modifications of nuclear proteins that occur during gene activation to determine if protein-nucleic acid interactions are important in gene modulation.

METHOD: Separation and characterization of nuclear proteins both histones and nonhistone proteins from control HeLa cell cultures and replicate cultures grown with sodium butyrate and hydroxyurea (two compounds that activate glycoprotein hormone production in HeLa cells).

FINDINGS: Histone proteins have been successfully extracted from purified HeLa cell nuclei and separated according to change utilizing high-resolution polyacrylamide gel electrophoresis. Cells have been radioactively labelled in control, sodium butyrate-treated, and hydroxyurea-treated cultures prior to fractionation, and nuclear protein modifications have been observed by autoradiography of dried gels following histone separation.

CLINICAL RELATIONSHIP: This area of research is relevant to understanding alterations in gene activity associated with abnormalities in development and differentiation and perhaps to the process of neoplasia.

311. REGULATION OF DIHYDROFOLATE REDUCTASE GENE EXPRESSION

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We are studying the regulation of dihydrofolate reductase (DHFR) gene expression in cultured mouse 3T6 fibroblasts. We have shown previously that the expression of this gene is very low in resting 3T6 cells. When the resting cells are serum stimulated to re-enter the cell cycle, DHFR gene expression increases dramatically at the beginning of S phase. We have isolated a methotrexate-resistant 3T6 cell line that overproduces the enzyme and its mRNA by a factor of 300, and that regulates DHFR gene expression in a manner similar (if not identical) to

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normal 3T6 cells. We are using this overproducing cell line to study in more detail the molecular mechanism(s) responsible for increasing and decreasing DHRF gene expression. We will attempt to answer the following questions: 1. Do the content and rate of production of DHFR mRNA increase at the beginning of S phase? 2. Is the production of DHFR mRNA controlled at the level of transcription or by regulating the efficiency of processing of the initial transcription product into mature mRNA? 3. Does the stability of DHFR and/or its mRNA change during transitions between the resting and growing state? 4. Are increases or decreases in DHFR gene expression controlled by regulating the efficiency of translation of DHFR mRNA? 5. Is DHFR gene expression regulated in the same manner (and perhaps by the same control signals) as other S phase enzymes?

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312. MOLECULAR MECHANISMS OF ANDROGEN MEDIATED GENE EXPRESSION IN MALE SEX ACCESSORY GLANDS

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The objective of this project is to study the mechanisms involved in androgen mediated gene expression in the rat prostate and the seminal vesicle. A major group of secretory proteins in both organs is under androgen control. Both organs have a high concentration of poly(A ion)-mRNA which code for this group of secretory polypeptides. The structural genes for these polypeptides have been prepared and cloned using the 'tailing method' and the plasmid pBR322. Many of the plasmids containing structural genes for these androgen dependent polypeptides have been identified by hybrid arrest translation. Large pieces of DNA containing the chromosomal gene for one of the seminal vesicle proteins (SVS IV) has been identified in a rat gene library and is presently being purified.

BIBLIOGRAPHIC REFERENCES: Mansson, P.E., Carter, D.B., Silverberg, A.B., Tully, D.B. and Harris, S.E.: Isolation and partial purification of the major abundant class rat seminal vesicle poly(A ion)-messenger RNA. Nucleic Acid Res. 7: 1553-1565, 1979. Silverberg, A.B., Tully, D.B., Mansson, P.E. and Harris, S.E.: In vitro translation of major abundant class poly(A ion)-mRNA from rat seminal vesicle. Arch. Androl. 4: 249-255, 1979.

313. MOLECULAR BASIS OF SPECIES DIFFERENCES IN LEVELS OF LYSOZYME GENE EXPRESSION

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Lysozyme genes as they exist in the genomes of a series of related organisms will be cloned by recombinant DNA methods and studied to compare the arrangement and potential evolutionary rearrangement of DNA sequences surrounding and intervening the coding sequences. Conserved sequences and/or sequence structures that have regulatory significance in gene expression may be discovered. The process of evolution will be examined at a fundamental level leading to an enhancement of our understanding of genome structure and function.

Initially a pair of ruminant species and a pair of primate species will be chosen for study. Ruminants have been discovered to exhibit an extremely high level of lysozyme gene expression in their stomach mucosa; primates on the other hand exhibit high levels of the enzyme only in their tears and milk. Gene rearrangements have been postulated to account for adaptive differences in species - it will be interesting to see if a rearrangement of elements can be observed in the gene structure of lysozyme in these different organisms.

314. GENE REGULATION IN MAMMALIAN CELLS

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The objective of this research proposal is to use SV40 as a simple model to study the basic mechanism of gene regulation of mammalian cells. We plan to: 1. Analyze the mechanism for the production of SV40 mRNA by studying the relationship between various post-transcriptional processing steps for the generation of mature mRNA. 2. Analyze the regulation of SV40 late gene expression during early or abortive infection by analyzing the rate of late mRNA synthesis and its subsequent processing. 3. Analyze the secondary structure of SV40 late nuclear RNA cross-linked in vivo in the search for structural basis of splicing.

315. COLLAGEN GENE EXPRESSION IN DIFFERENTIATING F9 CELLS

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Retinoic acid induces the differentiation of F9 embryonal carcinoma cells into parietal endoderm; a process which is enhanced by dibutyryl cAMP. This differentiation includes the induction of Type IV collagen gene expression. I propose to use mRNA for Type IV collagen to construct a cDNA clone in E. coli. This cloned DNA will be used as a hybridization probe to identify and quantify Type IV mRNA in differentiating F9 cells. This will allow the exploration of the level at which Type IV collagen gene expression is controlled by retinoic acid and dibutyryl cAMP. Specifically I will investigate whether this control is exerted at the level of RNA transcription, RNA processing, turnover of mature RNA, or translation of this RNA.

This investigation will be accomplished using the following procedures: 1) purification of Type IV collagen mRNA, 2) synthesis of a cDNA copy of collagen mRNA using reverse transcriptase, 3) cloning the collagen cDNA using the bacterial plasmid pBR322 and, 4) using the cloned cDNA as a hybridization probe to study the type of control that is operative in collagen gene regulation.

316. MECHANISMS AND REGULATION OF GENE EXPRESSION

Berg, P., Canaani, D., Nicholas, J., Okayama, H., Peabody, D., Sklar, J., Smith, A., Subramani, S., Fromm, M., Buchman, A., Stanford University, School of Medicine, Dept. of Biochemistry, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

The molecular biology of SV40. Our specific aims are to: a. determine what nucleotide sequences are involved in initiation and termination of early and late transcription, (i.e. the promoters and terminators) and what factors influence the selective expression of these transcriptional signals. b. determine what nucleotide sequences are involved in RNA splicing and, subsequently, the nature of the RNA substrates and enzymes needed for splicing. c. determine the function of leaders and splicing in mRNA biogenesis, particularly, if leaders and intervening sequences function in transcriptional or translational regulation. d. determine if the positioning and/or nucleotide sequence of structural genes and intervening sequences influence their processing and expression.

SV40 transducing vectors for study of gene expression. Our specific aims are to: a. develop an array of SV40-based vector-host systems for transducing specific genes into mammalian cells. Such vectors should be capable of being propagated as 1) virions 2) integrated elements in mammalian chromosomes and 3) autonomously replicating plasmids. b. use such recombinant genomes to explore a range of basic questions concerning mechanisms of gene expression e.g. transcription, termination and polyadenylation, splicing, capping and translation etc. (See specific aims la-d above.) c. construct transducing vectors whose maintenance can be selected for in appropriate cultured cells. d. introduce human and/or rabbit genomic and cDNA segments that code for normal and genetically altered forms of alpha-and beta globin-like proteins (e.g. alpha and IV (alpha-like) and beta, delta, gamma and epsilon (beta-like)) into vectors of the type mentioned above to correlate structural and devel-

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opmental parameters with their expression in the transduced cell. e. construct SV40 recombinant genomes containing rat genomic insulin gene I and the cDNA coding for human pituitary growth hormone to determine whether the precursor polypeptides are formed, processed properly and secreted in cultured cells.

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317. HORMONE REGULATION OF GENE EXPRESSION IN MAMMALIAN CELL

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In the rat pituitary tumor cell line GH3, the expression of the prolactin gene is stimulated by the sex steroid 17-beta-estradiol and the hypothalamic factor, thyrotropin releasing hormone (TRH). The expression of the growth hormone gene in this tumor line is stimulated by corticosteroids and thyroid hormones. At present, the quantitative changes in mRNA production coupled to hormonal induction are most readily explained by supposing that primary control lies with transcription. We will study the primary effect of hormones on gene regulation in these tumor cells by making a careful analysis of transcriptional units, their instantaneous rates of transcription, the formation of mRNAs from nuclear precursors and the metabolic fate of these mRNAs. These experiments utilize cDNA clones specific for growth hormone and prolactin RNA sequences. The chromosomal segments of these genes will also be isolated and cloned to facilitate a detailed analysis of transcriptional unit organization. The transcriptional units will be related to the sequences at the capped 5' end and poly-adenylated 3' end of nuclear and cytoplasmic RNA. These studies will provide criteria for the development of an authentic in vitro system of transcription, and will contribute to an understanding of regulation of gene expression in a hormone-responsive tumor cell line.

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318. GENE EXPRESSION IN NERVOUS VS OTHER TISSUES

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We propose to establish DNA clones whose RNA transcripts are found exclusively in the brain cytoplasm (brain-specific) and use them to strengthen our overall research project on the gene expression in the nervous system. The overall objective of this research is to understand why brain transcripts have an unusually large complexity compared with those of other tissues. For this, we study the transcriptional complexity (the fraction of rat genome expressed as RNA) of various rat tissues, tumors, and their derivative cell lines, with special emphasis on the nervous system. In order to make our research more incisive, we propose to isolate DNA clones which represent a portion of the genome specifically transcribed into messenger RNAs (mRNA) of the brain. DNA fragments of the entire rat genome have been amplified in a bacteriophage lambda, from which we will select those for brain mRNAs. We expect to isolate several classes of brain-specific DNA clones with different quantitative levels of transcription, i.e., rare, abundant, and intermediately abundant classes. These clones will be extremely useful in furthering our studies of gene expression in the brain and other organs.

BIBLIOGRAPHIC REFERENCES: Sueoka, N.. D.M. Chikaraishi, S.S. Deeb, P. Hseih, M. Imada and Y. Tomozawa (1979). Cell type conversion of a rat neural stem cell line in vitro and complexity of gene expression in rat brain. Proc. XIV Internat. Cong. Genetics, 1978, Moscow, in press.

319. REGULATION OF RNA POLYMERASE I GENE EXPRESSION

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The major objective of this research is to examine the regulation of expression of gene(s) for RNA polymerase I, the enzyme responsible for the synthesis of ribosomal RNA. This study will determine whether the alterations in enzyme activity observed in response to physiological stimuli are due to changes in the number of RNA polymerase I molecules or due to activation of preexisting enzyme. Antibodies to RNA polymerase I will be prepared and levels of RNA polymerase I will be quantitated by radioimmunoassay. Immunological techniques will also be used to ascertain whether RNA polymerase I contains any subunits in common with the other two nuclear RNA polymerases. A radioimmunoassay will be developed to measure individual subunits of RNA polymerase I. The levels of each subunit will be monitored under varying physiological conditions. Such studies will elucidate whether expression of all subunits is coordinately controlled or whether those subunits specific to RNA polymerase I can be regulated independently from the subunits RNA polymerase I has in common with RNA polymerase II and III. The immunological and structural properties of RNA polymerase I from a neoplasm (hepatoma) will be determined and compared to those of enzyme from normal tissue (liver). Such studies will delineate whether unique gene(s) for RNA polymerase I are expressed in neoplasia, a disease state characterized by increased production of ribosomal RNA. Finally, the levels of RNA polymerase I in normal liver and hepatoma will be measured under various dietary and hormonal conditions to determine whether RNA polymerase I levels in these tissues are regulated by similar physiological parameters.

320. DNA STRUCTURE AND GENE EXPRESSION IN NEOPLASTIC TRANSFORMATION

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This is part of a broader project. A summary of this subproject is not available.

321. ACCURACY IN GENE EXPRESSION

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The observed high accuracy in DNA replication and in gene expression cannot be explained on the basis of hydrogen bonding alone. On the level of translation the ribosome plays an active role in a mechanism of proofreading which favors incorporation of cognate transfer RNA over non-cognate transfer RNA. E. coli mutants displaying reduced misreading levels have been isolated during the previous project period. This phenotype has been correlated with increased release factor activity in one of the mutants studied. It remains to be determined whether the mutation involves the structural genes or an inhibitor of release factor activity. The former possibility would permit construction of an overproducer of release factor; the latter possibility identification and purification of an hitherto unknown inhibitor. Other mutants will be further analyzed by in vitro protein synthesis using Q beta RNA as template. A naturally occuring readthrough protein can be detected in this system. This study should reveal potential alterations in efficiency of initiation, elongation rate and readthrough, i.e., misreading at a terminator codon. Mutants with altered ribosomes will be further studied for possible changes in enzymatic binding of cognate and non-cognate transfer RNA. The role of GTP hydrolysis in ribosomal proofreading will be further elucidated using a non-hydrolyzable GTP analogue.

It is likely that alteration of the mechanisms responsible for maintaining accuracy of gene expression will have far-reaching consequences for cell physiology. A better understanding of these mechanisms in E. coli will be helpful in evaluating their

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possible role in eukaryotic cells, particularly with respect to aging and cancer.

322. GENE REGULATION AND CANCER

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The principal aims of the proposed studies are to elucidate the regulation and mechanism of gene transcription both in normal and in neoplastic cells; and to investigate changes in gene structure and gene regulation induced by carcinogens. These aims will be approached by physicochemical methods, particularly fast kinetics and nanosecond fluorescence spectroscopy.

As a first attempt, studies will be carried out with the bacterial system. The experiments include fluorescence probe studies of E. coli RNA polymerase and fast kinetic studies of the elementary steps involved in RNA synthesis. If these experiments prove fruitful, similar approaches will be applied to the mammalian system. Particular emphasis will be placed on the structure and interaction of RNA polymerase and chromatin in their biological environment, and the role of multiple forms of RNA polymerase in gene regulation.

The biochemical actions of a carcinogen, aflatoxin, will be studied. The possibility of a covalent linkage between aflatoxin and DNA will be explored. Equilibrium and kinetic studies will be carried out to reveal the structural changes of DNA induced by aflatoxin binding to correlate its biological activity with molecular structure. Furthermore, the effect of aflatoxin on the elementary steps involved in gene transcription will be examined.

323. CONTROL OF GENE EXPRESSION - NORMAL AND NEOPLASTIC

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The long range goal of these experiments is an understanding of the regulation of gene expression in immunoglobulin (Ig) producing mouse myeloma cells by characterizing cell lines which are mutant in their production of Ig.

The goals for the current year included the characterization of cell lines which had failed in the synthesis of either H or L chains or were synthesizing structurally altered H or L chains.

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324. CONTROL OF GENE EXPRESSION IN MAMMALIAN CELLS

Gefter, M. L., Massachusetts Inst. of Technology, School of Science, Dept. of Biology, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

The overall objective of our research is to define the enzyme-catalyzed reactions necessary for the biosynthesis of messenger RNA in eukaryotes. We have previously described two in vitro systems capable of supporting the synthesis of messenger RNA precursors of cellular and viral origin. An isolated nuclei system supports initiation capping, elongation, termination and polyadenylation of Adeno-virus mRNA precursors. We wish to modify and explore this system further in order to isolate the enzymes responsible for these reactions as well as for those needed for RNA splicing. A soluble system capable of catalyzing some of these reactions has been developed and it will be fractionated in order to establish the role of specific proteins in initiation of transcription as well as the role of specific DNA sequences acting as promoters.

The role of differentiation in governing transcription activity will be studied. Extracts capable of supporting pre-mRNA, biosynthesis will be prepared from cells expressing and not expressing immunoglobulin light chains. Promotor sequences will also be mapped for the mouse light chain genes.

325. MITOCHONDRIAL GENE EXPRESSION IN MALIGNANT CELLS

Clayton, D. A., Stanford University, School of Medicine, Dept. of Pathology, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

This proposal is concerned with the mechanism of gene expression of mouse mitochondrial DNA. The ribosomal DNA region of this genome has been shown to be actively transcribed and the ribosomal RNA genes are located in close proximity to the origin of heavy strand DNA replication. We are utilizing cloned mitochondrial DNA fragments for hybridization and sequencing experiments combined with RNA sequencing to analyze this common control region for initiation of DNA replication and transcription.

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326. REGULATION OF GENE EXPRESSION IN HUMAN CELLS

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The regulation of gene expression in mammalian cells is complex. Human fibroblast interferon production in poly(I).poly(C)-induced cultures of diploid human fibroblasts (FS-4 strain) is subject to a post-transcriptional (translational) repressor mechanism which appears to inactivate or degrade interferon mRNA. An inhibition of this repression by appropriate use of inhibitors of RNA (e.g. DRB, 5,6-dichloro-1-beta-D-ribofuranosyl-benzimidazole) and protein (e.g., cycloheximide) synthesis leads to a marked (50-fold) paradoxical enhancement of human interferon yields ('superinduction'). Increased interferon mRNA stability in superinduced cells is the major contributor to this phenomenon. We intend to: 1) Develop a cell-free system to investigate the regulation of human fibroblast interferon mRNA stability. This line of work should lead to the eventual biochemical characterization of the molecules involved in the post-transcriptional repressor mechanism. 2) Carry out further investigations of the synthesis, structure and function of human fibroblast interferon mRNA. We will investigate the size of the fibroblast interferon transcription unit, the regulation of transcription, and the role of 3'-terminal poly(A) in the appearance of cytoplasmic interferon mRNA and in its function, and determine the structure of the 5' end of interferon mRNA. 3) Develop improved procedures to enhance human fibroblast interferon yields from diploid cell cultures and evaluate the use of DRB as a superinducing adjuvant in vivo.

These investigations not only address fundamental questions in molecular biology but also should provide a sound basis for obtaining larger amounts of human fibroblast interferon that may be used in the clinic against a variety of viral and neoplastic diseases of man.

Investigations of the mechanism of action of DRB, an inhibitor of hnRNA and mRNA synthesis and also of the synthesis of adeno-, papova-, and oncornavirus RNA in mammalian and avian cells, represent a study of transcriptional regulation in human cells. DRB blocks RNA synthesis at multiple discrete sites within a few hundred nucleotides of the promoter in several of AD2-specific transcription units in HeLa cells. Furthermore, these sites appear to function even in the absence of DRB and lead to premature RNA chain termination. DRB is thus a probe for transcriptional attenuation. (Text Abridged.)

327. GENE ORGANIZATION AND EXPRESSION

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The long-term objectives of our research are to understand the organization and expression of regulatory and structural

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gene systems in simple eucaryotes, and to explain in molecular terms the function of the centromere in controlling chromosome segregation during mitotic and meiotic cell divisions. As a simple unicellular eukaryote, yeast is an excellent model system for studies on gene organization and regulation in higher cells. The methods we have developed over the past 3 years should now enable us to isolate and study any region of the yeast (or other simple eukaryote) genome, including centromeric DNA. Because of the lack of highly repetitive DNA sequences near the centromere of yeast chromosomes, we are able to isolate the centromeric DNA sequences by overlap hybridization techniques. Functional minichromosomes can be constructed, consisting of a yeast chromosomal replicator, a yeast centromere sequence, plus suitable genes for selection. These minichromosomes segregate as true chromosomes through mitosis and meiosis. Elucidation of the molecular events occurring during spindle formation and attachment of spindle fibers to chromosomes should now be possible. Current projects include: (a) determination of nucleotide sequence of yeast centromeres; (b) isolation and characterization of nuclear proteins binding specifically to centromeric DNA; (c) genetic studies on the behavior of minichromosomes in meiosis; and (d) functional and structural studies on yeast chromosomal replicators.

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328. REGULATION OF HUMAN GENE EXPRESSION

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The aim of this research project is to examine the molecular structure of DNA in Beta plus and Beta zero thalassemia. Beta plus thalassemia is a heterogeneous genetic disorder characterized by a decreased synthesis of Beta globin; Beta zero thalassemia is associated with absent Beta globin. Messenger RNA for Beta globin has been demonstrated to be present in decreased amounts in Beta plus thalassemia, and is either absent or abnormal in Beta degrees thalassemia. There is no large deletion of Beta globin gene sequences detected in either Beta degrees or Beta plus thalassemia. These observations suggest that the defect in these disorders is related to either defective transcription or abnormal processing of the Beta globin mRNA. The gene responsible for Beta thalassemia is closely linked to the structural Beta globin locus. Thus, it is reasonable to look for the molecular defect in Beta thalassemia DNA in either the Beta globin structural gene, intervening sequences, or the untranscribed regions flanking the structural gene in either the 5' or 3' direction. Use of the Charon 4A vector allows the complete purification of specific genes from the 1 to 10 million genes present in cell DNA. Charon 4A accepts large (8-22 kb) fragments of cellular DNA. It is, therefore, possible to isolate clones containing the entire Beta globin gene sequence, including the intervening sequence as well as the flanking, untranscribed DNA sequences. To contruct a library of clones of DNA, high molecular weight cellular DNA from patients with Beta thalassemia will be partially restricted with Eco RI, and fragments of approximately 20 kb in length will be selected. These fragments will be ligated to vector Charon 4A arms generated by Eco RI digestion. Recombinant DNA molecules will be packaged into infective phage particles. The library of recombinant phage will be amplified by propagation in E. Coli DP50SupF, and will be screened for Beta globin sequences by transferring phage DNA to nitrocellulose filters, and hybridizing to 32P-labelled specific Beta globin cDNA probe. The recombinant plaques positive for Beta globin sequences will be selected and propagated, and DNA will be isolated. This will be examined by restriction endonuclease digestion. (Text Abridged.)

329. SITE-DIRECTED MUTAGENESIS OF TRP OPERON LEADER SEQUENCES

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The objective of the present proposal is the assessment and identification of the role(s) of the tandem trp codons in the leader transcripts of the trp operons of E. coli and S. typhimurium. Of particular interest is the relationship of these codons to the ability of the attenuator to terminate transcription prematurely. The approach would entail: 1. Site-directed mutagenesis of cloned trp leader sequences to alter their trp coding capacities. The approach would involve the synthesis of mutant primers for DNA polymerase in vitro so that the replication in vivo would elicit the incorporation of mutant sequences into plasmids harboring trp leader sequences. 2. Scoring of the transcriptional behaviors of the resultant genes with respect to the abolition or retention of attenuator function.

With this experimental design nucleotides coding for the trp leader peptide could be substituted or frame-shifted. One could then assess the impact of the identities and positions of specific codons on attenuator function.

330. ANALYSIS OF GENE REARRANGEMENTS DURING MACRONUCLEAR DEVELOPMENT IN OXYTRICHA

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The summary for this project is under entry no. 28.

331. DNA STRUCTURE AND GENE REGULATION

Wells, R. D., Zacharias, W., Singleton, C., Kilpatrick, M., Klein, R. D., Goodman, T., Lambert, P., Stirdivant, S., Chapman, K., University of Wisconsin, Madison Campus, School of Agriculture & Life Sciences, Dept. of Biochemistry, *Agriculture Hall, Madison, Wisconsin*, 53706, United States of America

The summary for this project is under entry no. 10.

332. STUDIES OF RABBIT IMMUNOGLOBULIN ALLOTYPE GENES

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The organization and expression of rabbit immunoglobulin kappa chain constant region genes will be investigated by the use of cloning and nucleic acid hybridization techniques. Messenger RNA molecules coding for the region of the kappa light chain will be isolated from antibody producing cells expressing each of the four group b allotypes, b4, b5, b6, and b9. The cDNA transcripts of these molecules will be cloned in bacterial plasmids or bacteriophages and used as probes to test for the presence of unexpected C-kappa genes in the DNA of rabbits which are 'homozygous' for one of the allotypes. In this manner we hope to determine if the group b allotypes represent alleles at a single locus, or a closely linked family of duplicated genes with a polymorphic control mechanism which mimics a Mendelian pattern of segregation. Additional experiments with these probes and nucleic acid sequencing techniques should help to characterize the postulated control mechanism. The results of these experiments should have substantial significance for the genetics of the immune system.

333. REGULATION OF TRYPTOPHAN GENES IN N. CRASSA

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In order to begin to understand the regulation of tryptophan metabolism in eucaryotes, two genes involved in tryptophan metabolism in Neurospora crassa, (1) the tryptophan synthase gene (tryp-3), and (2) the kynureninase gene, will be cloned in E. coli and/or S. cerevisiae. Clones will be produced by dA:dT tailing of Neurospora fragments into a plasmid selected for high yeast transformation efficiency. The specific genes will be selected either by functional complementation or by immunoselection. The complementation tests to be employed are, respectively, (1) tests complementing tryptophan synthase-deficient host mutants; or 2) tests allowing anthranilate synthase-deficient host mutants to grow on medium lacking tryptophan but containing kynurenine. Isolated clone DNA will be restriction mapped. The clones will be used to isolate specific Neurospora mRNAs. Obtaining one or both of these mRNAs will

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then allow determination of both the strand from which the mRNA is transcribed and the start-point of transcription. The DNA regions 5' to the mRNA start-points will be sequenced and compared with known procaryotic trp regulatory sequences. The DNAs will also be used as probes for additional regulatory factors.

334. SYNTHETIC AND BIOLOGICAL STUDIES OF NUCLEIC ACIDS

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The principal long-range goal is the continued development of methods for the total synthesis of biologically functional genes and understanding of molecular mechanisms of gene control and expression.

The total research involved in this program can be categorized as follows: 1) Further development of the chemical methods for the synthesis of deoxyribonucleotides. 2) Structure-function relationships in the totally synthetic tyrosine tRNA gene. a. Mechanism of the initial recognition of the promoter region by the RNA polymerase. b. Formation of the stable DNA-RNA polymerase complex. c. The initiation of transcription by the RNA polymerase. 3) Synthesis of a variety of promoters with carefully designed alterations in nucleotide sequences will be used to study the transcription mechanism. 4) Cloning of the totally synthetic gene in suitable plasmids and vectors for work on structure-function relationships. 5) Studies of the DNA-membrane interaction in bacterial and mammalian synthesis. 6) Studies of the DNA-protein interactions using enveloped viruses such as PM2, vesicular stomatitis virus (VSV) and Sindbis virus.

335. RNA SYNTHESIS AND TRANSPORT IN MAMMALIAN CELLS

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The aim of the proposed research is to investigate certain aspects of the molecular mechanism of gene expression in mammalian and other eukaryotic cells. We propose to continue our studies of the organization of nuclear ribonucleoprotein complexes containing hnRNA (nhRNP) sequences, and the role these structures play in the correct processing and splicing of mRNA sequences. Our previous results suggest a structure for hnRNP in which the large RNA molecule is folded on distinct protein complexes to give a repeating 30S RNP substructure. Most hnRNA and mRNA sequences are bound to these 30S subcomplexes but certain distinctive RNA regions are exposed or complexed with proteins other than the relatively simple set of polypeptides found in the 30S particles; for example self-complementary, or double-stranded RNA regions appear to be exposed while nuclear poly(A) is contained in a discrete 15S substructure within hn RNP. We have obtained electrophoretic and immunological evidence that the simple set of polypeptides that constitute the 30S RNP-core are relatively conserved amongst vertebrate organisms. We now intend to investigate in more detail the chemistry of these proteins, use dissociation-reconstitution systems to analyze the specificity of protein:protein and protein:RNA interactions, and employ our existing antibodies and newly-prepared monoclonal antibodies to examine the physiology of hnRNP complexes. Variations in the stoichiometry and metabolic properties of hnRNP core proteins suggest that they may be significant factors in the modulation of growth and differentiation of normal and transformed cells.

BIBLIOGRAPHIC REFERENCES: Martin, T., Jones, R., and Billings, P. (1979). hnRNP core proteins: synthesis, turnover and intracellular distribution. Mol. Biol. Rep. 5:37. Martin, T., McMullen, M., and Shaw, P. (1979). Poly(A plus)mRNA sequences in free mRNP and polysomes of mouse ascites cells. Mol. Biol. Rep. 5:37.

336. THE MOLECULAR BIOLOGY OF MAIZE CONTROLLING ELEMENT SYSTEMS

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The ultimate objective of the proposed research is an understanding of the mechanisms by which controlling elements regulate gene expression in maize. The proposed approach is to gain access to controlling elements at the DNA level via isolation of segments of maize genomic DNA containing mutable alleles of loci which have been documented to come under control by these systems. Specifically, the proposed research will involve investigations of three maize loci (Bz (Bronze), Sh2 (Shrunken-2), and Wx (Waxy)), for which gene products have been previously identified by others. The experiments will involve 1) purification of the identified proteins encoded by these loci, 2) generation of antisera to the purified proteins, 3) isolation of populations of maize RNA containing the mRNAs for these proteins, 4) construction, identification, and isolation of recombinant clones containing double-stranded cDNA inserts homologous to these mRNAs, and 5) utilization of the DNA from each recombinant as a probe in the identification and isolation of segments of maize genomic DNA (eventually, also in recombinant form) containing various stable and mutable alleles of the three loci.

337. DNA TRANSCRIPTION CONTROL IN NORMAL AND CANCER CELLS

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Stereospecific protein-DNA interactions in nucleosomes and nucleosomal arrays of chromatin from primate cells (human, monkey) are studied from the point of view of identifying the structural elements in both the DNA sequences and protein components (histones, nonhistones) accounting for the phasing relationships previously reported. The influence of the experimental variables including ionic strength, temperature and type of nucleases employed in maintaining or destroying the phasing relationships and permitting their detection and detailed analysis will be explored by a variety of techniques, including direct DNA sequencing. The properties and possible functions of a complex family of related primate repetitive DNAs--the alphoid DNAs--are studied by a variety of molecular biological and recombinant DNA techniques to elucidate their role and significance in karyotypic and molecular rearrangements of the primate genomes and their possible consequences for gene expression. Special focus will be on family of interspersed alphoid sequences, the KpnI families, which may be as abundant in primate genomes as the Alu family. A working model suggests that these sequences may bridge the transcribed, coding sequences with which they seem to be associated, and the non-transcribed, highly repetitive sequences, throughout the Primate Order. The purification, properties and sites of DNA cleavage by a site-specific mammalian endonuclease will be studied with the goal of understanding the determinants of its site-specificity, and its possible role in site-specific recombinations or rearrangements that may occur in mammalian cells. The working hypothesis is that the endonuclease is not DNA sequence-specific, but may recognize DNA tertiary structure.

BIBLIOGRAPHIC REFERENCES: Maio, Joseph J., Brown, F. L. and Musich, P. R. (1981). Toward a molecular paleontology of primate genomes I. The HindIII and EcoRI dimer families of alphoid DNAs. Chromosoma, in press. Maio, J. S., Brown, F. L., McKenna, W. G. and Musich, P. R. (1981). Toward a molecular paleontology of primate genomes II. The KpnI families of alphoid DNAs. Chromosoma, in press.

338. REGULATORY MECHANISMS IN TUMOR BIOLOGY

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This program-project inquires into the basic mechanisms which regulate the replication and differentiation of normal and cancer cells. A combination of genetical, biochemical and cell biological approaches is used to address the following problems: 1. An elucidation of the mechanisms by which RNA polymerases bind to defined DNA templates and carry out a regulated transcription of specific genes. 2. The clarification of the role of gene structure and other gene products in the regulation of transcription. 3. A description of the molecular reactions by which primary gene transcripts are processed into functional messenger RNA species. 4. The identification of the components in nuclear envelopes and the definition of their roles in the

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regulation of nuclear function. 5. The identification of the active components of the DNA replicase system and a definition of the processes by which DNA-chromatin complexes are replicated. 6. The development of genetic systems in eucaryotes (i.e., Physarum polycephalum and mouse teratoma cells) for the study of the regulation of cell replication and differentiation.

BIBLIOGRAPHIC REFERENCES: Mueller, G. C., Kajiwara, K., Kim, U. H., and Graham, J. Proposed Coupling of Chromatin Replication, Hormone Action, and Cell Differentiation. Cancer Res., 38: 4041-4045, 1978. Mueller, G. C. and Kim, U. H. Displacement of Estradiol from Estrogen Receptors by Simple Alkyl Phenols. Endocrinology, 102: 1429-1435, 1978.

339. TRANSCRIPTIONAL CONTROL OF 5S RNA GENES IN XENOPUS LAEVIS

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The overall goal of this proposal is to elucidate the transcriptional control of 5S RNA gene expression in Xenopus, by reconstructing a defined transcription complex which will accurately transcribe these genes in vitro. Towards this end, I propose to isolate and characterize the 5S DNA nucleoprotein complex formed upon incubation of 5S DNA sequences in an oocyte nuclear extract. Next, factors required for accurate transcription of 5S DNA minichromosomes will be isolated from oocyte nuclei. The factors will be characterized as to physical properties and mechanisms of action as well as their association with 5S DNA or RNA polymerase III. Finally, the regulatory molecules which direct somatic cells to express only somatic-type 5S RNA while repressing oocyte-type 5S RNA synthesis will be isolated using the reconstructed transcription assay.

340. REGULATION OF TRANSCRIPTION

Wu, G., Emory University, School of Medicine, Dept. of Microbiology, *1364 Clifton Rd. N.E., Atlanta, Georgia,* 30322, United States of America

The object of this proposal is to study the regulatory mechanisms of transcription in human cells. Human adenovirus is chosen as the model system. Successful development of an in vitro functional assay for gene activity in my laboratory has made possible the study of the mechanism of interactions of RNA polymerases II and III plus necessary factors with adenovirus genes. This year I have located the control regions surrounded or within the 5.5S RNA gene by using the truncated DNA as templates in this system. The system has been further fractionated. A full activity can be restored by combining the necessary components. In addition to the RNA polymerase III system, three systems of RNA polymerase II were developed from wheat germ, calf thymus and human KB cells. Discrete transcripts were synthesized from restriction fragments of adenovirus DNA. The goals set for the coming year are isolation of factors, further locating the control regions on DNA by in vitro mutagenesis and characterization of RNA polymerase II transcripts.

BIBLIOGRAPHIC REFERENCES: Wu, Guang-jer (1980), Faithful Transcription of Adenovirus 5.5 S RNA Gene by RNS Polymerase III in a Human KB Cell-Free Extract, J. Biol. Chem. (in press)

III. RNA BIOCHEMISTRY

A. TRANSCRIPTION, PROCESSING, AND TRANSPORT OF HETEROGENEOUS NUCLEAR RNA

341. REGULATION OF HISTONE GENE TRANSCRIPTION

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The specific objective of this proposal is to prepare, from Xenopus laevis oocytes, a cell free system for transcription of cloned Xenopus histone gene sequences (class II, transcribed by RNA polymerase II). This is believed to be possible because: 1) isolaton of structural genes from eukaryotic DNA libraries has been achieved, 2) microinjection of cloned class I or class III genes into Xenopus oocytes has resulted in accurate transcription (correct initiation and termination) of the exogenous DNAs by the corresponding RNA polymerases, and 3) soluble systems prepared from oocytes are capable of transcribing class III genes, and these systems are yielding to further fractionation and analysis. Furthermore, the histone genes are actively transcribed in Xenopus laevis oocytes in vivo. Therefore, the components necessary for their transcription must be present and, like those involved in transcription of the class III genes, they may be present in considerable excess relative to the endogenous chromosomal genes. Thus, although accurate transcription of a class II gene has not yet been reported for any reconstituted system, the present approach appears highly promising and could yield results of broad applicability to other systems.

The methods used in this study include established techniques for isolation and mapping of eukaryotic DNA sequences, microinjection into X. laevis oocytes, the analysis of RNA transcripts, and the establishment and fractionation of crude cell free transcription systems. The microinjection studies and attempts to achieve accurate transcription in nuclear or cellular extracts will be pursued concurrently in order to identify the most promising approach. Analysis of newly synthesized transcripts and mapping of initiation sites will be done using specific 5' end labelling of this RNA with beta32P-ATP (or GTP) followed by a variety of mapping procedures, or by internal labelling in conjunction with the nuclease S1 mapping technique.

Should success in achieving accurate transcription be readily attained, then the emphasis would be switched to the individual components via fractionation of the system.

342. TRANSCRIPTIONAL ORDER IN EUKARYOTIC CHROMOSOMES

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We will analyze the transcription unit sizes for adult, murine hemoglobin alpha and beta chain genes by UV mapping. This technique bypasses the need for isolating the primary transcript, which would be impossible if processing were to start before the completion of the RNA chain.

Induce Friend's leukemia virus transformed cells (GM 86) with 2 percent DMSO, label nuclear RNA with 3H-uridine for 20-40 min immediately following UV irradiation at different doses. Isolate the RNA and quantify the fraction of mRNA homologous to the Hb alpha and beta messenger sequences. RNA will be quantified by hybridization to denatured, filter-bound plasmid DNA containing the murine alpha and beta mRNA sequences (clones obtained from the laboratory of Charles Weissmann).

UV sensitivities will be converted to DNA lengths using rDNA genes and specific mRNA size classes as internal standards. We will, further, analyze by restrictase cleavage and Southern blotting the chromosomal arrangement of IgG2b H and L genes in ethionine induced variants of MPC-11 which are defective in IgG synthesis. Nonproducing variants will be identified by direct fluorescent antibody staining fluoresceine isothiocyante conjugated anti Ig2b)

BIBLIOGRAPHIC REFERENCES: Giorno, R. and W. Sauerbier (1979) Effects of DRB on metabolism of heterogeneous nuclear RNA in mouse myeloma cells. IRCS Medical Science: Biochemistry; Cancer; Cell and Membrane Biology; Metabolism and Nutrition; Pharmacology. 7:202.

343. THE PROCESS AND CONTROL OF TRANSCRIPTION OF SV40

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Our primary objective is to gain a thorough understanding of the relation of simian virus 40 (SV40) chromatin structure to its function as a template for transcription and replication. We have previously shown that the intracellular SV40 chromatin is heterogeneous with respect to its ability to function as a template, with recently replicated ('new') viral DNA serving preferentially. We are examining the possibility that an interaction with capsid protein(s) is responsible for the diminished template

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activity. A comparison of the structures of 'new' and 'old' viral chromatin is being made with the use of various nucleases.

We have recently shown that histone-deficient SV40 chromatin cannot replicate without the prior and/or concomitant addition of histones. Further studies will be aimed at determining the basis for the requirement of histones in the process of viral replication.

344. RNA POLYMERASE AND CONTROL OF TRANSCRIPTION

Burgess, R. R., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

This is part of a broader project. A summary of this subproject is not available.

345. EFFECT OF ANTICANCER DRUGS ON THE SYNTHESIS AND PROCESSING OF NUCLEAR RNA

Glazer, R. I., Ritch, P. D., Hartman, K. D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Applied Pharmacology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The synthesis and processing of nuclear and cytoplasmic transfer RNA (tRNA), ribosomal RNA (rRNA) and messenger RNA (poly(A)RNA) will be studied in L1210 and Ehrlich ascites cells. The effect of the antimetabolites 5-fluorouracil, 5-azacytidine, dihydro-5-azacytidine, sangivamycin, tubercidin, formycin A, 8-Azaadenosine and cordycepin (3'-deoxyadenosine) will be examined on the methylation of rRNA and tRNA, as well as their synthesis to establish the relevance of these processes to the cytotoxicity of these agents.

BIBLIOGRAPHIC REFERENCES: Glazer, R.I. and Peale, A.L.: Comparison between the inhibitory activities of sangivamycin and thiosangivamycin on nuclear RNA synthesis in L1210 cells in vitro. Biochem. Pharmacol. 29: 305-310, 1980. Glazer, R.I., Peale, A.L., Beisler, J.A. and Abbasi, M.M.: The effect of 5-azacytidine and dihydro-5-azacytidine on nuclear ribosomal RNA and poly(A)RNA synthesis in L1210 cells in vitro. Mol. Pharmacol. 17: 111-117, 1980.

346. PROCESSING OF NUCLEAR RNA IN COLON CARCINOGENESIS

Augenlicht, L. H., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

We are currently screening a library of mouse genomic DNA cloned in the bacteriophage Charon 4A to identify sequences which are specifically expressed in mouse colon tumor (transplantable and induced by dimetylhydrazine) and not in the normal colon. The 32P probes of normal and tumor RNA have been prepared by chromatography of the RNA on oligo dT cellulose and reverse transcription of the poly A plus fractions. They are used for screening by the plaque-filter technique of Benton and Davis (Science 196, 180, 1977). To confirm that isolated sequences are relatively abundant in the tumor, but not in normal tissue, 32P end labelled poly A plus RNA from normal and tumor will be hybridized to the isolated cloned DNA bound to filters. Quantitative in situ hybridization of high specific activity cloned probes will also be used to investigate the specificity of the sequences and to follow their expression during chemical induction of colon tumors.

BIBLIOGRAPHIC REFERENCES: Augenlicht, L.H. (1978) Staphylococcal nuclease digestion of relatively stable RNA in the chromatin and nuclear ribonucleoprotein fractions of human carcinoma cells. J. Biol. Chem. 253:3035-3041.

347. INTERFERENCE OF AZOCARCINOGENS WITH RNA PROCESSING

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Carcinogenic amino azo dyes in the liver disrupt the processing of newly synthesized nuclear RNA and its transport into the cytoplasm. Our previous experiments have shown that these disruptions are the result of the interference of the azocarcinogen with the function of the proteins of nuclear ribonucleoprotein particles. The goal of the proposed three-year project is to isolate and characterize the proteins of these particles isolated from liver, and to identify changes in their properties and functions during the early stages of hepatocarcinogenesis. To identify the steps in RNA processing, which are blocked by azocarcinogen, different fractions of RNA (polysomal and non-polysomal cytoplasmic poly(A) positive and poly(A) negative RNA, nuclear poly(A) positive RNA, RNA bound to nuclear ribonucleoprotein particles) from livers of control animals and animals at different stages of azocarcinogenesis will be compared. The RNAs will be characterized by their complexity and the extent of secondary modifications. They will also be tested for the presence and distribution of sequences copied from repetitive DNA. The results of all analysis will be correlated. The function of individual proteins and the inhibition of this function by azocarcinogen will also be tested in an in vitro system. In this system we will try to reconstitute ribonucleoprotein particles fully active in RNA processing from proteins of normal and defective particles. The proposed experiments should identify azocarcinogen-induced changes in the protein population of nuclear ribonucleoprotein particles which lead to complete processing of nuclear RNA.

BIBLIOGRAPHIC REFERENCES: Patel, N.T., Folse, D.S. and Holoubek, V.; Release of Repetitive Nuclear RNA into the Cytoplasm of Liver of Rats Fed 3'-Methyl-4-dimethylaminoazobenzene. Cancer Research 39, 4460-4465, 1979.

348. PROCESSING OF INTERVENING SEQUENCES IN EUKARYOTIC RNA

Abelson, J., Engelke, D. R., University of California, San Diego Campus, Graduate School, Dept. of Chemistry, *P.O. Box 109, La Jolla, California*, 92037, United States of America

The goals of this project are to isolate and characterize the enzymes responsible for the basic nucleolytic and ligation reactions involved in removing intervening sequences from eukaryotic primary transcripts (splicing); in particular those transcripts produced by RNA polymerases II (pre-messenger RNAs) and III (pre-tRNAS, various small molecular weight cellular and viral RNAs). Purification of the soluble enzymes will proceed by a variety of standard techniques with the distribution of activities followed by the effects of fractions on unspliced or selectively cut, but not ligated precursor RNAS (pre-tRNAs and part of the polyoma virus T antigen precursor RNA containing the spliced regions). When the basic splicing reactions can be reconstituted from highly purified fractions, several types of study can be undertaken. The possibility that the two systems share some component(s) will be examined. 2) The exact nature of the RNA intermediates will be determined. 3) The levels and possible sequence specific multiplicity of enzymes in various cells will be determined. In addition, a search will be conducted for factors which modify the rate or specificity (as between two tRNAs or different splice points on the same streak of polyoma T antigen RNA) of the splicing reaction.

349. DEVELOPMENTAL STUDY OF THE SYNTHESIS AND PROCESSING OF SILKMOTH NUCLEAR RNAS DURING CHORIOGENESIS

Kafatos, F. C., Beltz, G. A., Harvard University, Cambridge Campus, School of Arts & Sciences, Dept. of Biology, *Cambridge Station, Cambridge, Massachusetts*, 02138, United States of America

A number of factors may influence the appearance of functional mRNA in the cytoplasm of a cell during development. These include the organization of the processing of completed transcripts. We propose to study the role of transcription and nuclear processing of RNA on the developmental program of choriogenesis in the silkmoth. Three sets of experiments will be conducted. 1) Analysis of sequences transcribed during the various stages of choriogenesis. Are particular chorion sequences transcribed only during a limited period of choriogenesis? Do the periods of transcription parallel the periods of translation? 2)Identification of the promoter regions for chorion RNA synthesis and analysis of the splicing patterns of these transcripts. Do transcipts coding for proteins of the evolutionary class or those expressed during the same periods of choriogenesis have similar splicing patterns? Do the locations of promoters or the processing of transcripts influence the appearance of functional

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mRNA? 3) Identification of possible stage specific RNA polymerase activities which can accurately transcribe chorion DNA in vitro.

350. STUDY OF RNA INITIATION AND PROCESSING IN ADENOVIRUS

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Transcription and RNA processing in eucaryotes must be controlled in part by nucleotide sequences around the startpoint of transcription and around the cleavage and splicing points in the RNA. To identify the functions of some of these regions of DNA utilized in expression of the late genes of adenovirus, I propose to insert fragments of adenovirus-5 DNA into a cloning vehicle. The effects of the insert will be monitored by analyzing RNA products coded by genes downstream from the insert. The cloning vehicle will be an adenovirus-2 - adenovirus-5 derivative with a single cleavage site for a given restriction enzyme, contained within the nonessential region, and deletions amounting to approximately 10% of the genome. The fiber structural gene lies immediately downstream from the nonessential region. Therefore, analysis of the structure of nuclear and cytoplasmic fiber RNA products from cells infected with the recombinant will detect whether the insertions are directing additional transcription and/or processing. The initial insertion will extend from a few hundred base pairs upstream from the putative startpoint of late transcription through two of three segments normally spliced together at the 5' end of mature late mRAs.

351. DEVELOPMENTAL STUDY OF THE SYNTHESIS AND PROCESSING OF SILKMOTH NUCLEAR RNAS DURING CHORIOGENESIS

Beltz, G. A., Harvard University, Cambridge Campus, Biolegical Lab., Dept. of Biology, *16 Divinity Ave., Cambridge, Massachusetts*, 02138, United States of America

A number of factors may influence the appearance of functional mRNA in the cytoplasm of a cell during development. These include the organization of the template DNA, location of promoters, existence of specific RNA polymerases, and processing of completed transcripts. We propose to study the role of transcription and nuclear processing of RNA on the development program of choriogenesis in the silkmoth. Three sets of experiments will be conducted. 1) Analysis of sequences transcribed during the various stages of choriogenesis. Are particular chorion sequences transcribed only during a limited period of choriogenesis? Do the periods of transcription parallel the periods of translation? 2) Identification of the promoter regions for chorion RNA synthesis and analysis of the splicing patterns of these transcripts. Do transcripts coding for proteins of the same evolutionary class or those expressed during the same periods of choriogenesis have similar splicing patterns? Do the locations of promoters or the processing of transcripts influence the appearance of functional mRNa? 3) Identification of possible stage specific RNA polymerase activites which can accurately transcribe chorion DNA in vitro.

352. SYNTHESIS AND PROCESSING OF AVIAN RETROVIRUS RNAS

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Integrated proviruses of avian retroviruses are expressed by nuclear transcription into RNA which is processed, transported to the cytoplasm and translated. While the synthesis and processing of viral mRNAs are apparently accomplished by normal host enzymes, it is unclear whether the promoter(s) for synthesis intiation are located within the provirus or in neighboring host DNA. Our studies are designed to delineate the pathway of virus-specific RNA production with emphasis on the sites of RNA synthesis initiation. Employing cloned DNA segments and molecular hybridization, newly synthesized virus-specific RNAs will be identified and purified. The kinetics of appearance of potential precursors and intermediates as well as the mature RNAs, will be determined in pulse-chase studies. The purified RNAs, will be analyzed by nucleic acid hybridization, oligonucleotide 'fingerprinting' and sequencing techniques to characterize and locate the sites at which synthesis is initiated and processing occurs.

353. RNA PROCESSING IN HUMAN CANCER AND VIRUS-INFECTED CELLS

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This is part of a broader project. A summary of this subproject is not available.

354. STRUCTURE AND FUNCTION OF RNA PROCESSING SIGNALS

Zinder, N., Rockefeller University, Viral Oncology Lab., *66th & York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

355. STUDY OF THE METABOLIC AND STRUCTURAL CHARACTERISTICS OF THE HETEROGENEOUS NUCLEAR RNA IN HUMAN LEUKEMIC CELLS

Torelli, U. L., Torelli, G., Narni, F., Ferrari, S., Donelli, A., Franchini, G., Universita degli Studi di Modena, Inst. of Medical Clinics, *Via del Pozzo, c/o Policlinico, 41100 Modena, Italy*

OBJECTIVE: The investigation is aimed to explore the existence of structural and metabolic differences between heterogeneous nuclear RNAs (hnRNA) extracted from normal and leukemic cells.

APPROACH: Experiments were carried out to study the kinetics of hybridization in vitro to human DNA of hnRNA extracted from three types of cells: normal human promyelocytes, normal human lymphoblasts and leukemic human promyelocytes of the HL-60 cell line. In the labeled hnRNA from the two normal cell types, at least 15% of the sequences are complementary to reiterated sequences, and at least 25% are complementary to single copy sequences. In the labeled hnRNA from HL-60 cells, the least squares computer analysis indicates that at least 30% of the sequences are complementary to reiterated DNA sequences. Furthermore, the analysis did not suggest the presence of a class of sequences significantly corresponding to the single-copy sequences observed in hnRNA from normal cells. It is concluded that the increased repetitiousness of the heterogeneous nuclear RNA in the HL-60 cells is a leukemia-related characteristic of the cells.

PROGRESS: Several lines of research have been suggested by the findings reported above. Priority has been given to the direct determination of the complexity of the hnRNA from normal and leukemic cells. Such determination is now in progress.

356. STUDIES ON BIOSYNTHESIS, STRUCTURE AND FUNCTION OF MICROBIAL RNA

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Bacterial mutants defective in the biosynthesis of 5-methyluridine (m5U), 1-methylguanosine (m1G) and 5-methylaminomethyl-2-thiouridine (mam5s2U) in the tRNA have been studied. The genes trmA and trmD, which are responsible for the formation of m5U and m1G, respectively, have been cloned. The DNA fragment which harbors the trmA gene is only 1300 basepairs long, while the trmD gene has been found on a 4300 basepair DNA fragment. The trmA gene is located close to the argH gene (at 88 min. on the E. coli chromosomal map) and the trmD gene is close to the tyrA gene (at 56 min.). The trmC gene, which is responsible for the formation of mam5s2U in the tRNA, is 70% co-transducible with aroC (at 50 min.). The cloned DNA fragment will be used for promoter studies, since we have shown that these tRNA methyltransferases have at least wo modes of regulation. The results of this research are of interest in view of the high levels found in tumor cells of both the modified nucleoside in the tRNA and of the corresponding enzyme. Furthermore, we have discovered a novel link between the biosynthesis of aromatic amino acids and tRNA modification.

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357. ORGANIZATION OF DNA IN CHROMATIN

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OBJECTIVE: To study the way in which DNA is organized and constrained topologically in mitotic and interphase chromosomes.

APPROACH: Whole mount chromosomes viewed with the electron microscope have yielded relatively little information about the path the DNA molecule takes through the chromosome, largely because the product of the DNA-histone association is a highly compacted structure. Our approach is to study the organization of DNA liberated from histone depleted chromosomes spread on a water surface by the Kleinschmidt technique. We examine chromosomes released from mitotic cells lysed in high salt and non-ionic detergent, a method known to preserve higher order structure of DNA. These techniques are also being used to study the organization of normal and damaged DNA in various stages of the cell cycle.

358. MECHANISMS OF CONTROL OF PROTEIN AND RNA SYNTHESIS IN NEOPLASTIC GROWTH

Bernelli Zazzera, A., Del Monte, U., Perin, A., Scalabrino, G., Universita degli Studi di Milano, Istituto di Patologia Generale, *Via L. Mangiagalli 31, 20133 Milan, Italy*

OBJECTIVE: To detect metabolic changes in tumor cells.

APPROACH: Measurements of RNA synthesis, t-RNA levels, enzymes of polyamine synthesis and degradation in dividing cells (tumorous and non-tumorous).

359. REGULATION OF POLYOMA VIRUS DNA AND RNA SYNTHESIS STUDIED BY THE CONSTRUCTION AND ANALYSES OF VIRAL MUTANTS

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OBJECTIVE: We study how polyoma virus regulates RNA and DNA synthesis after infection of permissive and semi-permissive cells.

APPROACH: The study is carried out by construction and analyses of mutations in regulatory elements and early genes of the virus.

PROGRESS: During 1979, two classes of mutants were studied. The first class had a duplication of DNA sequences at the origin of DNA replication. These mutants appear to overproduce early gene products, at least before the onset of viral DNA synthesis. They also have a very rapid increase of the amount of viral DNA. We conclude that the 250 base-pair duplication contains the origin of DNA replication and, in addition, DNA sequences that code for a transcriptional promotor of early RNA synthesis.

The second class of mutants has deletions in the middle of the early genes, in sequences coding for middle- and large T-antigens. These mutants are viable. One of them, however, has a reduced ability to stimulate the metabolism of the host cell. It is also unable to transform rat cells.

360. STRUCTURE OF NUCLEAR RNA AND MESSENGER RNA FORMATION

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Knowledge of the mechanisms employed by animal cells in regulating the production of cytoplasmic messenger RNA (mRNA) is fundamental for an understanding of development and how the factors which control normal cellular growth are altered in the cancerous state. It is now established that at least some cellular mRNA as well as mRNAs of the DNA tumor viruses are processed from a larger nuclear precursor RNA. We have been able to separate HeLa cell mRNAs into four classes of molecules: a) those containing Poly(A) sequences (Poly(A plus)) as well as Oligo(U) sequences (Oligo(U plus)) (4-13%); b) Poly(A plus) Oligo(U minus) (52%); c) Poly(A minus) Oligo(U plus) (2%); d) Poly(A minus) Oligo(U minus) (33%). These classes of mRNAs have been characterized with regard to size. It appears that the Oligo(U) is in an intramolecular duplex with the 3' Poly(A). The location of the Oligo(U) in the mRNAs will be determined with particular interest in whether the Poly(A plus) Oligo(U plus) mRNAs are in a circular configuration that may be under distinct translational control. The different mRNA classes will be translated in vitro to allow further identification.

Also it will be determined whether the drug DRB effects the transcription or processing of hemoglobin mRNA in Friend cells. An understanding of these processes may reveal whether they are modified to alter the supply of mRNA according to growth or development requirements or changed when cells are transformed by DNA tumor viruses. Transformation is known to involve the integration of viral DNA into the host genome and the production of viral mRNA. It is not known how the presence of the viral mRNA(s) causes a loss of control of cellular growth, however, recent data indicate that the production of at least one of the host mRNAs coding for a large membrane protein is reduced.

361. DIHYDROFOLATE REDUCTASE RNA LEVELS DURING THE CELL CYCLE

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The general goal of this project involves the elucidation of the molecular mechanisms in control of dihydrofolate reductase synthesis in mammalian cells. Dihydroflate reductase is an enzyme required for the synthesis of nucleic acid precursors, and is the target for a number of agents employed in cancer chemotherapy such as methotrexate. Quantitation of primary gene transcripts and gene products during the cell cycle should reveal if transcriptional, processing, or translational factors (or a combination of these factors) are somehow responsible for the differences in dihydrofolate reductase levels observed at various stages of the cell cycle.

The development of resistance to methotrexate in human tumor cells and in several types of murine cancer has posed problems concerning the clinical usefulness of the drug. The availability of cells resistant to methotrexate in which the genes for dihydrofolate reductase are selectively amplified should allow the isolation of relatively large amounts of messenger RNA and enzyme per cell; comparison of these methotrexate-resistant cells and normal cells may provide information concerning the mechanism of selective gene amplification in drug insensitivity.

362. CONTROL OF RNA SYNTHESIS IN EUKARYOTIC CELLS

Penhoet, E. E., University of California, Berkeley Campus, School of Letters & Science, Dept. of Biochemistry, *Berkeley, California,* 94720, United States of America

The overall goal of our research is to elucidate molecular mechanisms which are involved in the regulation of RNA synthesis in mammalian cells. To approach this goal, we are using animal viruses as agents to specifically alter cellular RNA metabolism and as model RNA synthetic systems.

Infection of appropriate cells with picornaviruses results in a rapid inhibition of the initiation of RNA synthesis by RNA polymerase II. This inhibition is accomplished by a mechanism which does not result in gross alterations of either the enzyme or the template chromatin. Our future studies of this regulation will involve analysis of the expression of specific genes and specific proteins (particularly non-histone chromosomal proteins) in picornavirus infected cells. Much of the protein analysis will focus on determination of the patterns of specific modification of nuclear proteins, particularly phosphorylations.

The expression and replication of influenza virus genes are related and regulated processes which involve the action of a limited number of cellular and viral components. The goal of our studies in this system is to determine the manner in which these components act and interact in infected cells. Since phosphorylation of viral proteins seems to play a role in this system, the source and significance of this modification will be explored in detail.

363. CONTROL MECHANISMS IN HUMAN TUMOR CELLS - SMALL RNAS

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We are studying some abundant, long-lived, homodisperse, low molecular weight nuclear RNA species of unknown func-

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tion, particularly RNA species and D, in cultured human cervix carcinoma cells. C and D RNA exhibit several interesting characteristics: their transcription units may be up to 25 times larger than their known precursors; they seem to pass through the cytoplasm for a few minutes shortly after their transcription; and they appear to be present in heterogeneous nuclear RNA-protein particles. In addition, apparently about 5% of the cellular C and D RNA content is covalently linked to a low molecular weight subpopulation of chromatin DNA. Finally, RNA species C and D have 5'-end cap structures that are very similar to cap 2 of eukaryotic messenger RNAs, and D RNA markedly inhibits translation of mammalian messenger RNA in a cell-free extract.

Several types of experiments will be performed, toward the long-term objective of identifying the physiological role of these RNA species. First, through some in vivo experiments we wish to examine if D RNA might be involved in the inhibition of protein synthesis that occurs during mitosis. Another goal is to test if RNA species D and C are translated. A third aim is to study the dynamic relationship between the covalently-linked short DNA-small RNA complexes and a) the metaphase and interphase stages of chromatin, b) the known nucleoplasmic small RNAs, and c) bulk chromatin DNA. Finally, we wish to examine by microscopic autoradiography the intracellular behavior of purified labeled low molecular weight RNA species, after they have been microinjected into cultured human cells.

364. REGULATORY NUCLEOTIDES AND RNA SYNTHESIS

Pao, C. C., University of Mississippi, Medical Center, School of Medicine, Dept. of Biochemistry, *2500 N. State St., Jackson, Mississippi*, 39216, United States of America

The stringent control of RNA synthesis in E. coli will be investigated by studying the effect of ppGp, a nucleotide recently isolated (Pao and Gallant, J. Biol. Chem., in press). RNA produced by cells in the presence and absence of ppGp will be analyzed. rRNA synthesis will be measured by gel electrophoresis and DNA-RNA hybridization competition techniques using DNA prepared from lambda transducing phages carrying rRNA genes as templates.

An in vitro assay system will be developed to examine the formation of ppGpp. The precursor-product relationship will be established between ppGp and ppGpp, and the genetic element that controls the conversion between them will be investigated. Appropriate mutants will be isolated. An in vitro ribosomal system prepared from both wild type and mutant strains carrying temperature sensitive translational factors will be employed to study the metabolism of guanosine polyphosphates.

The genetic element that causes the failure in producing ppGpp in yeast will be defined. In addition, the possible effect of ppGpp on the synthesis of yeast ribosomal proteins will be studied.

Since the similarities between stringent control in prokaryotic cells and pleiotypic response in eukaryotic cells have provided an opportunity to understand the control of growth in mammalian cells, the very mechanism affected by the malignant transformation, the possible existence of regulatory nucleotides in eukaryotic cells will be investigated. This will be done with the aid of boric acid gel column chromatography and a new solvent system.

365. RNA METABOLISM IN DEVELOPMENT AND HORMONAL REGULATION

Key, J. L., Baulcombe, D., Nagao, R. T., Schoffl, F., Kroner, P., University of Georgia, Franklin College of Arts & Sciences, Dept. of Biochemistry/Botany, *Athens, Georgia*, 30601, United States of America

The poly(A)RNAs of control and auxin-treated soybean hypocotyl tissue have been shown by saturation hybridization to tracer-labeled single copy DNA to consist of similar populations within the limits of resolution (app. 5%) representing some 40,000 (1400 nucleotide long) different sequences. cDNA to poly (A) RNA hybridization showed, however, that auxin markedly alters the relative concentration of some abundant poly(A)RNAs. This result was confirmed and extended by in vitro translation in a wheat germ system of the poly(A)RNAs followed by 2-D gel fractionation of the translation products and by hybridization of poly(A)RNA to cloned sequences of double stranded cDNA. A detailed analysis of those sequences which are auxin-responsive will be made including relative synthetic rates, turn-over rates, relative concentration between nuclei and polyribosomes, developmentally and auxin-regulated poly(A)RNA. A soybean 'gene bank' will be screened for selected ones of the auxin-regulated gene sequences and the structure/ organization of the genes determined.

Additionally, rDNA will be cloned and the cloned sequences used for affinity chromatography purification of RNA polymerasel, promoter characterization and regulatory studies on RNA polymerasel modulation during tissue proliferation.

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366. SYNTHESIS OF HETEROGENEOUS NUCLEAR RNA AND POLY A IN ISOLATED NUCLEI

Fefer, A., University of Washington, Institutional Research Grant Committee, Dept. of Medicine, *1131 14th Ave. S., Seattle, Washington*, 98144, United States of America

OBJECTIVE: To test the hypothesis that RNA synthesized in isolated rat liver and Novikoff tumor nuclei under conditions of high ionic strength is primarily undergraded HnRNA, and to characterize the RNA thus formed with regard to poly A content.

APPROACH: Incubate nuclei (prepared from animals treated with 14C-RNA precursors) with 3H-labeled nucleotides to study both degradation of 'old' RNA and synthesis of 'new' RNA, and to isolate the RNA by phenol procedures and nucleic acid hybridization methods, with characterization by degradation by specific nucleases and chromatography.

PROGRESS: The prelabeled RNA is degraded to 5'-nucleotides (trapped in vitro by phosphorylation to triphosphates) at the same time as other new RNA is being synthesized from triphosphates. The nucleases responsible for the degradation have been characterized elsewhere. The poly A regions do not protect against this degradation.

367. RNA SPLICING - REMOVAL OF THE INTERVENING SEQUENCES

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In the past two years many investigators have shown that eukaryotic genes are interrrupted by noncoding regions called intervening sequences or introns. Evidence has been obtained showing that these intervening sequences are transcribed and subsequently removed during RNA maturation. The main objectives of this research project are 1) to isolate the enzyme(s) responsible for the removal of the intervening sequences in the 15S beta-globin mRNA precursor and 2) to investigate the mechanism of splicing.

An assay will be devised in order to isolate the enzyme(s) from mouse Friend cells. Nuclear RNA isolated from DMSO treated mouse Friend cells will be used as substrate. Specific processing of the 15S beta-globin mRNA precursor will be detected by resolving the RNA on agarose gels after incubation with various cell fractions, and measuring the decrease in the 15S globin mRNA species.

The mechanism of splicing will be investigated by 1) using site-specific mutagenesis to alter the nucleotide sequence at the intron-exon junction, 2) analysis of the intron after removal in order to determine if splicing can occur at multiple sites, and 3) determining the importance of secondary structure of the precursor in the splicing reaction.

368. RNA METABOLISM IN MAMMALIAN CELLS

Furth, J. J., Bello, L. J., Kelley, R. I., University of Pennsylvania, School of Medicine, Dept. of Pathology & Laboratory Medicine, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

The proposed work is designed to elucidate RNA metabolism as it is related to growth, development, and function of both normal and neoplastic cells. Emphasis will be placed on

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the study of RNA polymerase II and RNA polymerase III (C) (ribonucleoside triphosphate; RNA nucleotidyl-transferase (EC 2.7.7.6)), two of the three eukaryotic RNA polymerases.

RNA polymerase II has been shown to be responsible for mRNA synthesis, while RNA polymerase III has been shown to be responsible for the synthesis of 5S RNA, tRNA, and 5.5S adenovirus associated RNA. Using enzyme purified from calf thymus and bovine lymphosarcoma, RNA synthesis will be studied using both DNA and chromatin templates. In vitro studies utilizing purified enzyme and template should furnish an insight into the relative roles of polymerase and the various components of chromatin in determining the specific transcription which occurs in vivo. Emphasis will be placed on transcription of histone genes by RNA polymerase II and transcription of the 5S RNA and tRNA genes by RNA polymerase III. Specific transcription will be monitored with plasmids containing the genes for histones, 5S RNA, and tRNA.

369. RNA SYNTHESIS IN REOVIRUS-INFECTED MOUSE FIBROBLASTS

Gomatos, P. J., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

370. CHANGES IN RNA METABOLISM AND THE INDUCTION OF TUMORS

Apirion, D., Ray, B., Roy, M., Ray, A., Washington University, School of Medicine, Dept. of Microbiology & Immunology, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

The long-term objectives of this research program are: to understand the processes which determine differential gene expression, and to assess the contribution of disturbed patterns of gene expression in the onset and maintenance of malignancy. These studies will focus on small stable RNA molecules which are found in the nucleus. The specific goal of these studies is to assign functions to small stable RNA (ss RNA) molecules. This objective will be pursued by a combination of physiological, biochemical and genetical analyses.

In order to assess the function of the ssRNA molecules in the onset of malignancy, an attempt will be made to induce tumors chemically in the mouse liver and to follow the metabolism of ssRNA in the liver of these animals. The distribution of the genes corresponding to ssRNA in the mouse genome will be studied by nucleic acid hybridizations on gels. We shall determine whether ssRNA is more abundant in active or inactive chromatin, and to what extent it is part and parcel of chromatin. In order to assess whether the metabolism of ssRNA is affected in human tumors, fragments of tumors will be grafted to nude mice in which the metabolism of ssRNA will be studied.

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371. REPLICATION AND MODIFICATION OF MDV-1 RNA

Kramer, F. R., Columbia University, College of Physicians & Surgeons, Dept. of Medicine, *630 W. 168th St., New York, New York*, 10032, United States of America

This is part of a broader project. A summary of this subproject is not available.

372. RNA METABOLISM IN TUMOR VIRUS SV40-INFECTED CELLS

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The process of eukaryotic RNA metabolism in cultured cells infected with the DNA tumor virus SV40 will be investigated. An in vitro system will be established to probe the role of template structure, and host and viral protein factors in transcription. RNA sequence analysis will be applied to precisely map the positions of viral RNA transcription and processing, including cleavage and splicing. The role of RNA sequence and structure in metabolic regulation will be investigated. These studies should lead to new insights into the relationship between tumor virus and host, and into the basic process of eukaryotic RNA metabolism.

373. STRUCTURE AND FUNCTION OF VIRAL RNA

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This is part of a broader project. A summary of this subproject is not available.

374. RNA PRIMERS IN SV40 DNA REPLICATION

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The existence of RNA primers on about 30-60% of both long and short (Okazaki fragments) nascent DNA chains is now reasonably well established by data from subcellular as well as intact cell systems. Synthesized de novo at replication forks, their initiation does not depend on a unique DNA sequence signal and they are removed from nascent DNA chains before Okazaki fragments are joined.

Proposed in the project are experiments designed to extend our understanding of the role of RNA primers in the normal process of chromosome replication and to use this information to design proper assays for the enzymes and cofactors responsible. Specifically the experiments envisaged would be an attempt to answer the following questions: 1) Are RNA primers required for the initiation of Okazaki fragments during SV40 DNA replication, and, if so, how are they synthesized and excised? 2) How does DNA polymerase alpha specifically interact with native replicating chromosomes to allow the synthesis and completion of Okazaki fragments?

These questions will be posed in relation to simian virus 40(SV40) because SV40 provides us with a small, well defined, DNA genome that can continue 'normal' replication in either isolated nuclei or soluble nuclear extracts. The structure, composition and mechanism of DNA replication in these viral chromosomes is strikingly similar to that of the host. Since all of the substrates, templates and products of viral DNA replication are easily isolated and characterized, SV40 provides us with a homogeneous population of mammalian 'replicons' analagous to those found in mammalian chromosomes.

375. CONTROL, AT THE GENE LEVEL, OF THE SYNTHESIS OF STABLE RNA'S

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No summary has been provided to the Smithsonian Science Information Exchange.

376. RNA REPLICATION PICORNAVIRUSES

Semler, B. L., State University of New York, Health Science Center at Stony Brook, School of Basic Health Sciences, Dept. of Microbiology, *Stony Brook, New York*, 11790, United States of America

The research project that I propose is a study of picornavirus RNA replication, specifically in poliovirus. I will try to determine the role, if any, of the genome-linked protein, VPg, in poliovirus RNA replication. The approach to be taken will be to attempt to isolate an in vitro replication system from poliovirus-infected cells that is capable of RNA chain initiation. The RNA products of this in vitro system will be characterized as to whether: 1) They are present as part of replicative intermediates, 2) They contain VPg linked to their 5' termini, 3) They are newly-initiated RNA chains or merely add-on products, 4) They hybridize back to 'plus' or 'minus' strand poliovirus RNA. An attempt will then be made to determine the nature, if any, of the interaction of VPg with the structural polypeptides that comprise the polio replicase complex and how this small protein might serve as an 'initiator polypeptide' in lieu of the 5' nucleoside triphosphate usually found to initiate RNA synthesis.

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377. APPLICATION OF RNA SEQUENCE SPECIFICITY AND FRAGMENTATION METHODS TO THE BIOLOGY OF REOVIRUS

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Mammalian reovirus whose genome consists of 10 structurally and functionally discrete double-stranded RNA(dsRNA) segments provides a useful model system for study of fundamental biological processes, for example, mechanisms of virus-host interactions such as cell and tissue specificity, viral morphogenesis, and analysis of events which affect the cell function in particular and the intact organism in general. It is both appropriate and feasible to address questions posed by substantial genetic and biochemical lines of investigation through direct RNA sequence analysis. Specifically, I propose: 1) to determine the sequence of approximately 300 nucleotides from the 5' and 3' termini of each of the 10 dsRNA genome segments of reovirus serotype 3; 2) determine secondary structural features in end termini of all genomic segments of serotype 3, S1 gene of serotypes 1, 2 and 3, and several deletion mutants by enzymatic methods; 3) employ RNAse T1 mapping to determine whether deletion mutants contain altered primary RNA structures; 4) determine the complete sequence of the S1 gene of reovirus serotypes 1, 2, 3; and 5) apply the sequence information generated from the above experiments to confirm genetic assignments of viral proteins to their respective genomic segments.

Currently I am developing methods analogous to DNA restriction cleavage to break large RNA molecules into sizes convenient for sequencing and continuing development of a direct RNA sequencing method utilizing base-specific RNAses and polyacrylamide gel electrophoresis.

378. AFLATOXIN B1 AND NUCLEOLAR RNA SYNTHESIS

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Aflatoxin B1, produced by certain strains of Aspergillus flavus, is a potent liver carcinogen for many animal species including, possibly man. Recent studies from this laboratory have demonstrated this carcinogen, when injected into rats, strongly inhibits rat liver nucleolar RNA synthesis (J. Biol. Chem. 252, 3245, 1977). Furthermore, it was found that the enzyme, RNA polymerase I, which transcribes the nucleolar chromatin was not affected by this carcinogen. In order to understand the mechanism of this inhibition, other alternatives should be considered: a) aflatoxin B1 may interact with nucleolar DNA thus inpairing its template function, or b) aflatoxin B1 may react with one of the key regulatory proteins on the nucleolar chromatin and, as a result, the expression of the nucleolar gene is greatly inhibited. It is the aim of this proposal, therefore to determine which one of the above mentioned alternatives is the true mechanism of action of aflatoxin B1 in the inhibition of nucleolar RNA synthesis. In order to determine whether the nucleolar DNA template is the source of inhibition, experiments will be carried out using RNA polymerase I from control group to transcribe a) isolated total nucleolar DNA, b) enriched rDNA gene, c) purified rDNA gene from both control and aflatoxin B1 treated groups, to see if the DNA template function from aflatoxin B1 treated group is indeed impaired. In order to determine whether nucleolar chromosomal proteins are directly involved in the aflatoxin B1 inhibition, nucleolar histones, nucleolar High Mobility Group proteins, and other nonhistone proteins are selectively extracted from control and aflatoxin B1 treated nucleolar chromatin. These proteins from each group are then used in step-wise fashion to reconstitute nucleolar chromatin with identical nucleolar DNA from the control source. RNA polymerase I isolated from control group will be used to transcribe the chromatin template at each stage of the reconstruction, thus to ascertain if, indeed, certain groups of proteins from the aflatoxin B1 treated group produces clear differential inhibitory effect on the otherwise normal nucleolar DNA template. The protein involved can be identified by further purification.

379. HNRNA IN NORMAL AND TRANSFORMED CELLS

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Measurements of the amount of steady-state hnRNA and parameters characterizing its size distribution may be of value in understanding the biological properties of malignant cells. The populations of hnRNA ffound in several transformed and normal 3T3 cells and one normal and transformed human fibroblast line were compared. Uncompleted studies indicate that although the human and murine cells have characteristically different hnRNA populations, the normal and transformed cell lines are very similar.

380. SMALL NUCLEAR RNAS IN BOMBYX MORI

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Since their discovery in 1968, small nuclear RNA molecules (snRNAs) have been characterized in a variety of eukaryotic organisms but their function remains unknown. Recent evidence suggests that these molecules may play a role in regulating gene expression by controling messenger RNA processing in the nucleus. We propose to investigate the function of these molecules in the silkworm Bombyx mori, an organism ideally suited for studies concerning the molecular control of developmental and regulatory processes. First, snRNAs will be isolated and characterized from various Bombyx tissues. Individual snRNA species will be identified by 2-D gel electrophoresis, fingerprinting and sequencing. Next, genomic clones of DNA coding for snRNA species will be directly constructed using radioactive snRNA probes. Third, cloned DNA probes complementary to snRNAs of interest will be used to detect variations in relative rates of synthesis or accumulation of different snRNAs in developing Bombyx tissues. Finally, snRNA interactions with specific Bombyx messenger RNA species will be analyzed in vivo and in vitro. Aminomethyltrioxsalen/UV crosslinking will be used to join intermolecular complexes involving fibroin-mRNA precursor and mature message with their associated snRNAs.

B. MESSENGER RNA

381. CHARACTERIZATION OF CDNA CLONES DERIVED FROM THE ALPHA AND BETA GLOBIN MESSENGER RNA SEQUENCES OF ADULT XENOPUS LAEVIS (ABBREV)

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The DNA copies of the messenger RNA sequences encoding the two major adult globin polypeptides of Xenopus laevis, the South African Clawed Toad, have been isolated by molecular cloning in a bacterial plasmid. The globin sequences contained in these two recombinants have been shown to be almost full length copies of the mRNA sequences. By determining the DNA sequnce of the 3' terminal region of the cloned sequences it has been possible to determine the C terminal amino acid sequence of the globin polypeptides encoded by the mRNA sequences in the clones. There is sufficient homology in the C terminal region of the known globin polypeptides to identify one of the cloned sequences as an alpha globin mRNA and the other cloned sequence as a beta globin mRNA. The complete 3' non-coding region of both mRNA sequences has also been determined. In the case of the beta globin mRNA, a considerable degree of homology with mammalian beta globin sequences was found in the region between the poly A and the sequence AAUAAA (a sequence found close to poly A in all known eukaryotic polyadenylated RNA sequences). The two cloned sequences have been used as hybridization probes to analyze the nuclear transcripts of the globin genes in immature blood cells. The nuclear transcripts are larger than cytoplasmic globin mRNA and the sizes of the alpha and beta globin RNA transcripts and presumed processing intermediates are very similar to those found in mammalian blood cells. In the case of the

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alpha globin gene this result has been confirmed by 'Southern mapping' of genomic DNA.

382. PURIFICATION AND CLONING OF THE MOUSE PLASMINOGEN ACTIVATOR MRNA

Reich, E., Belin, D., Rockefeller University, Lab. of Chemical Biology, *66th & York Ave., New York, New York*, 10021, United States of America

This proposal aims to detect and purify the mRNA of mouse cellular plasminogen activator (PA). The isolation of this mRNA will lead to the synthesis and cloning of a cDNA probe required for studying the mechanisms and regulation of PA synthesis. PA clones will also help in analysing the organization of this gene in cellular DNA.

In a clone isolated from 3T3-MSV cells, PA accounts for 0.2% of total protein synthesis. The secreted PA has been purified and used to raise anti-PA antibodies. In vitro translation of PA mRNA in reticulocyte lysates will be assessed by immuno-precipitation of the translation products. To isolate PA mRNA, membrane-bound polysomes will be precipitated by anti-PA antibodies. After synthesis of cDNA by reverse transcriptase, the DNA will be inserted into a plasmid vector. Identification of PA clones involves hybrid arrested translation. To increase the proportion of PA sequences in cloned DNA, specific oligonucleotides will be used as primers for reverse transcription. Cloned DNA and homologous sequences in the mouse genome will be compared by hybridization of restriction digests. In parallel, conditions for the selection of cell lines which overproduce PA will be devised.

383. STRUCTURE OF IMMUNOGLOBULIN MESSENGER RNAS AND GENES

Adams, J. M., Core, S., Kemp, D. J., Webb, E., Gough, N. M., Walter & Eliza Hall Inst. for Medical Research, Dept. of Biochemistry & Biophysics, *c/o Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia*

The aim of our project is to learn more about the structure and organization of immunoglobulin genes and mRNAs in the mouse. To this end, we have cloned a number of kappa mRNAs and different heavy chain mRNAs and characterized the clones. We are using probes made from these clones to investigate the number and arrangement of Ig genes in the germline state and in various Ig-synthesizing cells. In particular, for the heavy chain system, we are investigating the mechanism of VH-C mu joining by analysis of a cloned active C mu gene and the mechanism of CH switching. Analysis of other clones within the CH locus is underway.

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384. STRUCTURE AND FUNCTION OF SNRNP

Jeanteur, P., Assens, C., Brunel, C., Lelay, M. N., Liautard, J. P., Sri Widada, J., Centre Paul Lamarque, Laboratoire de Biochimie, *2 Ave. Bertin Sans, B.P. 5054, 34033 Montpellier, France*

OBJECTIVE: Study of the possible involvement of snRNA and associated proteins in mRNA splicing.

APPROACH: 1) To identify and characterize snRNP proteins. 2) To look for snRNP-associated enzymatic activities. 3) To study the antigenic determinants of snRNP recognized by autoimmune antibodies.

PROGRESS: HeLa cell snRNP has been extensively purified from the complex hnRNP containing heterogenous nuclear RNA.

385. ORIGIN AND STRUCTURE OF THE LATE ADENOVIRUS 2 MRNA LEADER

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Spliced mRNAs derived from noncontiguous genomic regions have been observed for several eukaryotic genes. In adenovirus 2 a leader sequence originating from three parts of the genome is joined to one of at least twelve different late mRNAs. This organization of adenovirus 2 late mRNAs raises several important questions about the function and synthesis of the leader, several of which could be answered by sequencing the leader.

I propose then to determine the leader sequence for a late mRNA (100K mRNA). 100K mRNA will be purified and will serve as a template for AMV reverse transcriptase. Short restriction endonuclease fragments complementary to the 5'-terminus of 100K specific mRNA will be isolated and used to prime the synthesis of DNA complementary to the entire leader sequence. The rapid DNA sequencing technique developed by Gilbert and Maxam will then be used to determine the cDNA sequence.

I also plan to sequence those nucleotides surrounding each of the three leader components in the adenovirus 2 genome. Restriction endonuclease fragments containing nucleotides both surrounding and including each part of the leader sequence will be isolated and sequenced. These studies should be valuable in assessing potential functions for the leader such as ribosomal interaction and capacity for translation, and also in analyzing late mRNA maturation, in particular the role of leader-neighboring sequences in precursor mRNA processing events.

386. THE ROLE OF MRNA STRUCTURE AND ITS ROLE IN THE REGULATION OF GENE EXPRESSION

Paterson, B. P., Rosenberg, M., Queen, C. L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Developmental Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The role of mRNA structure in the efficiency of translation has been under investigation utilizing defined prokaryotic mRNAs as templates in a eukaryotic protein translational system. Structural features within the mRNA which are important for efficient ribosome binding and translation have been studied.

387. STRUCTURE AND FUNCTION OF MODIFIED NUCLEOSIDES OF RNA

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Our aim is to understand the structure-function relationship of modified nucleosides in nucleic acids, tRNA, rRNA and mRNA. Our objective is to establish, by x-ray diffraction techniques, the details of the stereochemistry of modified nucleosides and mRNA caps. We will investigate the details of the stereochemistry of codon-anticodon interaction, the reading of the genetic code and the roles played by modified nucleosides. We will develop stereochemical criteria that will be of some help in developing a rational chemotherapy program involving modified nucleosides, cytokinins and protein synthesis inhibitors that are analogs of mRNA caps and the 'two-five A's'. We will investigate the intercalation of water and ions between nucleic acid bases and the consequences of such intercalation in nucleic acid structure and function.

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388. STRUCTURE OF TRANSCRIPTION UNITS IN MAMMALS

Tahsu, M., Wolgemuthjarash, D., Rockefeller University, Graduate School, Dept. of Molecular & Cell Biology, *66th & York Ave., New York, New York*, 10021, United States of America

The elucidation of how mRNAs are formed from nuclear transcripts is integral to the understanding of the regulation of gene expression. Recent investigations demonstrating the composite nature of late Ad-2 and SV-40 mRNAs have led to questioning the generality of this phenomenon. In particular, are mammalian cellular mRNAs also mosaic molecules? That is, do mammalian cytoplasmic mRNAs contain sequences at their 5' termini which are not complementary to the DNA sequences

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adjacent to those which code for the structural gene? Electron microscopic analysis of RNA:DNA hybrid molecules will be used to determine regions of sequence complementarity between a specific mRNA and a long fragment of DNA containing the mRNA sequences. The experiments will involve isolation of a pure mRNA using a cloned cDNA as an affinity probe. DNA will be obtained from cloning long CHO cell DNA fragments in lambda vectors after extensive gene enrichment or from already existing long fragment-containing clones.

389. STRUCTURE AND EXPRESSION OF THE PROLACTIN GENE

Kawasaki, E. S., Sloan Kettering Inst. for Cancer Research, Cellular Gene Expression & Regulation Lab, *410 E. 68th St., New York, New York*, 10021, United States of America

Prolactin messenger RNA will be isolated from a clonal strain of rat pituitary tumor (GH4) cells. Complementary DNA synthesized from this mRNA will be used in the formation of a hybrid bacterial plasmid containing a prolactin structural gene sequence. The hybrid plasmid will be used as a probe in determining the structure of the natural prolactin gene in the rat genome, using restriction enzyme mapping methods. The prolactin gene structures found in normal rat liver and pituitary and rat pituitary tumor cells will be compared in order to determine whether the gene structure has changed during organ development and/or oncogenic transformation of the cells. The prolactin probe will also be used to investigate the existence of a nuclear RNA precursor for mature prolactin mRNA. Pulse labelled nuclear RNA will be fractionated on denaturing sucrose gradients, and then hybridized to prolactin plasmid DNA immobilized on nitrocellulose filters. These results will be compared with the results of studies on the size and structure of the prolactin gene. If time permits, the hormonal regulation of the synthesis and processing of prolactin mRNA will be studied by means of quantitative RNA-DNA hybridization techniques, using highly labelled prolactin plasmid DNA as the probe.

390. GENE ORGANIZATION AND MRNA FORMATION IN MAMMALIAN CELLS

Jelinek, W. R., Rockefeller University, Lab. of Molecular Cell Biology, *66th & York Ave., New York, New York*, 10021, United States of America

This proposal is divided into two sections. The first section describes experiments to investigate the nature of inverted repeated DNA sequences in mammalian genomes (ir-DNA) and a low molecular weight RNA hydrogen-bonded to poly(A)-terminated heterogeneous nuclear RNA (hnRNA) and messenger RNA (mRNA), and to determine their positional relationships to messenger RNA coding sequences. The ir-DNA sequences are transcribed into heterogeneous nuclear RNA where they account for approx. 25% of the hnRNA mass; some are also present in polyribosomal, poly(A)-terminated RNA. On the average, each mole of poly(A)-terminaed hnRNA contains approx. one mole of the low molecular weight RNA hydrogen bonded to it. Experiments are also described to investigate the nature of DNA replication origins and to identify them in cloned DNA fragments from the Chinese hamster ovary cell genome. Also described are experiments to ligate the thymidine kinase gene to various DNA fragments of the CHO genome to increase the frequency of transformation of TK negative to TK positive cells either by increasing the frequency of integration of TK into the host cell genome or by replication of the TK gene as a unit independent of the host cell chromosomes, i.e., as a mini-chromosome.

The second section described experiments to investigate two enzymatic reactions known to play a role in mRNA production and maintenance in eukaryotes, RNA:RNA splicing and end addition to and shortening of poly(A) in mRNA molecules. We have developed an in vitro RNA:RNA splicing reaction that correctly breaks and rejoins RNA molecules to produce mRNAs from their precursor RNA molecules. This system will be purified further to obtain the active components. We have isolated the cytoplasmic poly(A) polymerase from HeLa cells. This enzyme is absolutely dependent on divalent cations, has a mol. wt. of 75,000, and apparently is also a poly(A) endonuclease, leaving a product of approx. 15-18 AMP residues with a 5' pAp and a 3' A-OH. We plan to further purify and characterize these two enzymatic activities as well as to determine whether the poly(A) binding protein, which also has a mol. wt. of 75,000, is in fact the same protein.

391. REGULATION OF MRNA ABUNDANCE

Getz, M. J., Moses, H. L., Mayo Foundation, *200 1st St. S.W., Rochester, Minnesota*, 55901, United States of America

Mechanisms which control the frequency distribution of polyribosome-associated messenger RNA sequences are being studied in a parent and chemically-transformed derivative line of AKR mouse embryo cells in culture. Molecular cloning techniques are being utilized to derived a number of individual cloned sequences corresponding to mRNAs present in differing intracellular frequency in the two cell types. These mRNAs include transcripts of endogeneous AKR-murine leukemia virus (MuLV)-related genes, alpha and beta globin genes, and randomly selected genes coding for unknown products. These cloned sequences will then be utilized in molecular hybridization experiments to measure the relative nuclease sensitivity of the corresponding genes in isolated chromatin, and the relative rates of synthesis, processing, and cytoplasmic metabolism of each corresponding mRNA sequence. The results of these experiments whould allow an evaluation of the relative contribution of transcriptional and post-transcriptional mechanisms in the regulation of gene expression and may provide insight into the lesions in these mechanisms which accompany chemical transformation of cells in culture.

BIBLIOGRAPHIC REFERENCES: Siegal, S.P., Hodgson, C.P., Elder, P.K., and Getz, M.J. (1980) Polyadenylate-deficient Analogues of Poly(A)-containing mRNA Sequences in Cultured AKR Mouse Embryo Cells. J. Cell. Physiol. 103:417-428. Ono, T., and Getz, M.J. (1980) Levels of Ovalbumin Messenger RNA Sequences in Nonoviduct Tissues of the Chicken. Dev. Biol. 75:481-494.

392. REGULATION OF SLIME MOLD MESSENGER RNA SYNTHESIS

Jacobson, A. S., University of Massachusetts, Medical School at Worcester, School of Medicine, Dept. of Microbiology, *55 Lake Ave. N., Worcester, Massachusetts*, 01605, United States of America

I propose to purify the Dictyostelium messenger RNA for actin, isolate the genomic DNA sequences coding for actin, and characterize actin mRNA transcription in vivo as well as in isolated nuclei and in cell-free systems programmed with purified actin DNA. I intend to define the structure of the actin gene and the nature of all polypeptides and co-factors responsible for its correct and regulated expression.

393. RNA AND GROWTH CONTROL IN ANIMAL CELLS

Darnell, J. E., Powell, D., Georgieff, M. S., Chen Kiang, S., Bourque, L., Krauter, K., Rockefeller University, Lab. of Cell Biology, *66th & York Ave., New York, New York*, 10021, United States of America

This project deals with the molecular details of messenger RNA biosynthesis -- the definition of transcription units in mammalian cells, the correspondence between these transcription units and mRNAs formed from them -- and the potential steps along this complicated pathway where regulation of mRNA supply is effected.

The use of recombinant DNA clones and specific segments of Adenovirus DNA has allowed the suggestion that regulation occurs both at the level of transcription and mRNA stabilization.

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394. NORMAL AND VIRUS TRANSFORMED CELLS - MRNA BIOGENESIS AND GENETIC REGULATION

Darnell, J. E., Rockefeller University, Lab. of Molecular Cell Biology, *66th & York Ave., New York, New York*, 10021, United States of America

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This research is concerned primarily with nucleic acid metabolism in mammalian cells featuring studies on the biogenesis and metabolism of messenger RNA. Growing cells transformed by viruses as well as cells infected by DNA tumor viruses are being studied with the major effort devoted to the details involved in the transcription and conversion of large nuclear RNA molecules into functional polysomal messenger RNA and the cytoplasmic stability of the mRNA. The long-range goal is to understand the regulation of mRNA production in mammalian cells since this must be a pivotal point of regulation in cell growth.

395. REGULATION OF MRNA PRODUCTION

Ziff, E., Rockefeller University, Viral Oncology Lab., *66th & York Ave., New York, New York,* 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

396. SYNTHESIS OF MRNA (MICE)

Greenberg, J., Worcester Foundation for Experimental Biology Inc., *222 Maple Ave., Shrewsbury, Massachusetts,* 01545, United States of America

This is part of a broader project. A summary of this subproject is not available.

397. BIOSYNTHESIS OF ADENOVIRUS EARLY MRNAS

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Mutations will be introduced by enzymatic and chemical methods into localized regions at the left end of the Adenovirus 5 genome. In separate experiments mutagenesis will be directed to the 5' and 3' ends of the early region encoded there (early region 1, the transforming region of Ad5) and into areas where sequence is joined in spliced mRNAs from this early region. Host range mutants generated by these methods will be selected by their ability to grow on 293 cells, a human cell line transformed by Ad5 which expresses early region 1 functions, and their inability to grow on HeLa cells. mRNA- mutants will be isolated by screening the generated host range mutants with the sensitive and simple S1 mapping method to identify mutants which fail to induce early mRNAs in HeLa cells. mRNA- mutants will be mapped by marker rescue and sequenced. An adaption of the S1 mapping procedure to the analysis of pulse labeled transcripts will be used to characterize the biochemical defect in these mutants. By extensively mutagenizing localized regions throughout early region 1, it should be possible to identify all steps in mRNA production which require specific DNA and RNA sequences and to define these sequences. Determining the sequence of mutations which revert the mRNA- mutants will yield insight into the mechanisms of these steps.

The host range mutants obtained in these studies will be placed into complementation groups and representatives of each complementation group will be assayed for their ability to transform rat embryo and hamster embryo cells. Such studies will define the Ad5 genes required for transformation by Ad5.

398. REGULATION OF MITOCHONDRIAL MRNA IN TUMOR CELLS

Avadhani, N. G., University of Pennsylvania, School of Veterinary Medicine, Dept. of Animal Biology, *39th & Pine, Philadelphia, Pennsylvania,* 19104, United States of America

OBJECTIVE: Study the altered genetic regulatory controls in some mouse tumor cells by extensive characterization of purified mitochondrial mRNAs. It is also proposed to study the genetic origin(s) of mitochondrial mRNA in normal and tumor cells.

APPROACH: Mitochondrial mRNA will be purified from the organelle specific polysomes and their physical, chemical, biological properties and their genetic origins will be determined.

399. REGULATION OF MRNA IN XENOPUS LAEVIS EMBRYOS

Sargent, T. D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Biochemistry, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

I propose to monitor the behavior of approximately 100 different genes that are actively expressed in embryonic Xenopus laevis cells. The primary analytical technique will be to hybridize individual cloned mRNA probes selected at random from a Xenopus gastrula cDNA clone library (Dworkin, M.B. and Dawid, I.B. (1980) Dev. Biol. 76, 435-448) to 'blots' of electrophoretically fractionated RNA isolated from the cytoplasm and nucleus of embryos of several developmental stages. This experiment will establish the extent to which mRNA concentrations are regulated during frog embryogenesis, and will also provide considerable information regarding the mechanisms involved in this control.

Gene regulation at the level of transcript processing is expected to be prominant and will be given special attention. Genes displaying particularly interesting modulation during development can be isolated from a genomic library and studied in detail.

400. CONTROL OF MESSENGER RNA EXPRESSION IN DEVELOPMENT

Infante, A. A., Wesleyan University, School of Liberal Arts & Sciences, Dept. of Biology, *Middletown, Connecticut,* 06457, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

401. CONTROL OF BETA-ENDORPHIN AND DYNORPHIN MRNA SYNTHESIS IN PITUITARY CELLS

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Beta-endorphin is an endogenous opiate peptide found in the human brain. Administration of this peptide to laboratory animals results in behavior associated with the intake of opiates. Injection of beta-endorphin also produces dramatic motor effects and increases the pain threshold in laboratory animals. Dynorphin is also an opiate peptide found in the brain but is clearly different from beta-endorphin. It is 50 times more potent than beta-endorphin and, thus, may be an important opiate itself.

The major aim of this research is to better understand how synthesis of beta-endorphin and dynorphin is regulated in the anterior and intermediate lobes of the pituitary and the hypothalamus, and to determine how glucocorticoids and exogenous opiates affect these processes.

To this end, I will clone cDNA synthesized from an RNA template coding for beta-endorphin and dynorphin. This cDNA will be sequenced in order to determine gene structure and organization. This sequence information will allow us to determine if dynorphin is synthesized as part of a large precursor protein containing other neuropeptides, as is the case for beta-endorphin, or as a single product.

The cDNA for beta-endorphin and dynorphin will also be used as a hybridization probe to determine: 1) What regions of the brain transcribe beta-endorphin and dynorphin genes. 2) If beta-endorphin and dynorphin specific mRNA is processed in the nucleus. 3) The effects of glucocorticoids and opiates on transcription or these genes.

402. ENZYMATIC ACTIVITIES MEDIATING ENDONUCLEOTLYTIC MATURATION OF PRECURSOR TO MOUSE BETA-GLOBIN MRNA IN FRIEND CELLS (ABBREV)

Dierks, P. M., Universitat Zurich, Institut fuer Molekularbiologie, *Winterthurerstrasse 266A, 8057 Zurich, Switzerland*

The DNA sequence encoding the mouse beta-globin polypeptide is separated into two non-contiguous regions by an intervening DNA sequence of 550 base pairs in length. The intervening sequence is transcribed into a precursor RNA but subsequently removed during maturation of the precursor to the 10S beta-globin mRNA by a process known as RNA splicing. It is the objective of this project to demonstrate that RNA splicing will occur in vitro and to subsequently purify and characterize the enzymes involved. Minimally, a site-specific endoribonuclease and an RNA ligase activity are believed to mediate this process.

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To demonstrate RNA splicing in vitro 32P-labelled RNA will be synthesized in nuclei isolated from DMSO-induced Friend cells and fractionated by rate zonal centrifugation. The distribution of beta-globin specific sequences will be determined by filter hybridization to beta-globin PCR1 hybrid DNA. In order to provide a suitable supply of substrate for enzyme purification procedures, a beta-globin gene will be reconstructed in vitro in such a way that it can be isolated and used as a substrate for E. coli DNA-dependent RNA polymerase. Purification of the RNA splicing enzymes will be effected by standard fractionation techniques using the synthetic precursor as a substrate. In addition dsRNase and RNA ligase assays will be performed to quantitate specific activities.

403. IMMUNOGLOBULIN MRNA AND GENES IN T CELL TUMORS

Kemp, D. J., Harris, A. W., Cowman, A. M., Walter & Eliza Hall Inst. for Medical Research, Molecular Biology Laboratory, *c/o Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia*

A key unresolved question in molecular immunology is the nature of the antigen receptors on thymus-derived lymphocytes (T cells). Recently we used cloned immunoglobulin (Ig) heavy chain mu constant region (C mu) DNA sequences to demonstrate that the C mu gene is expressed as several distinct mu RNA species in mouse thymocytes and in some cultured T cell tumor lines. Our objective is to characterize the T cell mu RNA species, and to analyze rearrangements of the genes encoding them in order to define their relationship to T cell receptors. We will use recombinant DNA technology to construct cloned cDNA copies of the T lymphoma mu RNAs and cloned chromosomal genes. Nucleotide sequence studies on the cloned DNAs should reveal whether the RNAs contain variable regions similar to those which function in B cells. To define which classes of T cells express Ig genes we will examine highly purified normal T cell populations and immunologically functional T cell lines. To gain an insight into the control of Ig gene expression in T and B cells we will construct a variety of T lymphoma cell hybrids and analyze Ig RNA expression in them. We will use a similar recombinant DNA approach in order to ask whether some cells of the granulocyte-macrophage series display immunological diversity, a surprising question raised by our unexpected observation that mu RNA species are present in most myeloid tumor cell lines.

These studies should contribute to an understanding of the molecular basis of cell-mediated immune mechanisms and to an understanding of the control mechanisms which result in developmentally distinct transcripts.

404. CONSTRUCTION AND CHARACTERIZATION OF RIBOSOME BINDING SITE MUTANTS IN THE BACTERIOPHAGE PHI X174

Smith, M., Russell, D. W., University of British Columbia, Dept. of Biochemistry, *6174 University Blvd., #300, Vancouver, British Columbia*, V6T 1W5, Canada

The mechanism by which ribosomes recognize specific initiation sites in prokaryotic messenger RNAs (mRNAs) has been an area of extensive research during the past several years. Two regions of nucleotide sequences in the mRNA have been found to be of importance in this process. They are a region of complementarity between bases on the 5' side of the initiator codon and the 3' end of the 16 S ribosomal RNA (rRNA), and the initiation codon itself. The purpose of the proposed research is to study the effect of mutations in these sequences believed to be important in the correct initiation of protein synthesis. Using the recently developed techniques of site-specific mutagenesis, we will construct defined mutants of the bacteriophage phi X174 whose transcribed mRNA differs in its theoretical ability to bind to ribosomes. Initially we will contruct and characterize a mutation in the ribosome binding site of the gene G protein of phi X174. The effect of this mutation on the G mRNA's ability to initiate translation correctly will be studied in vivo and in vitro.

405. BIOGENESIS OF MESSENGER RNA

Edmonds, M. P., Wood, W. M., Kopp, D. W., Wallace, J. C., University of Pittsburgh, School of Arts & Sciences, Dept. of Biological Sciences, *4200 5th Ave., Pittsburgh, Pennsylvania*, 15260, United States of America

The long range objectives of this work are to increase the understanding of the biogenesis of messenger RNA (mRNA) in mammalian cells. Our approach has been based on the fact that many, but not all, RNA molecules in the heterogeneous nuclear RNA (hnRNA) and in the cytoplasmic mRNA populations contain common structural features, as is the case for the 3' and 5' termini. Other distinctive structural features in sub-fractions of these RNA populations include: (1) a transcribed oligo A sequence in hnRNA molecues that lack poly A, that is also not found in cytoplasmic mRNA that has also been detected in about 10 percent of the mRNA molecules of HeLa cells.

We have recently developed a technique that allows the isolation of the oligo U(plus) mRNA in good yield that depends on the removal of the poly A sequence before hybridization to poly A attached to agarose. The oligo U (plus) mRNA contains both cap 1 and cap 2 species, an observation confirming its cytoplasmic location since cap 2 structures are not found in nuclear RNA. We are examining the translatability, sequence complexity and homology with total mRNA, of the oligo U containing mRNA as well as the location of the oligo U sequence within selected size classes of the oligo U(plus) mRNA.

We are investigating RNA processing that includes characterization of transcription, polyadenylation, capping and RNA ligation reactions observed in mouse myeloma nuclei. An RNA ligase-like activity detected in these nuclei that ressembles in its reaction mechanism the RNA ligase present in E. coli after infection with T4 bacteriophage is being characterized.

406. ISOLATION OF NUCLEAR PRECURSORS TO VITELLOGENIN MRNA

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Accumulating evidence suggests that messenger RNA (mRNA) is derived from a larger initial transcript which is somehow processed within the nucleus into the final cytoplasmic product. At present there is no satisfactory method for separating nuclear mRNA precursors from bulk cellular RNA. The ability to isolate specific mRNA precursors would permit detailed investigations into the nuclear processing steps necessary for the production of mRNA. The central purpose of the proposed research is to provide such a method using a novel labeling technique.

The system we have chosen for this problem is the estrogen-primed rooster. Livers of these animals synthesize large amounts of the egg-precursor protein, vitellogenin, as well as large amounts of its mRNA. Newly synthesized nuclear liver RNA will be separated according to density from bulk RNA by labeling transcripts in single-cell suspensions with ribonucleosides substituted with the heavy atoms 13C and 15N. The size of the vitellogenin mRNA containing sequences will be identified within size fractionated newly synthesized RNA using a purified complementary DNA transcript. Processing of these transcripts into mature mRNA will be analyzed using a pulse-chase protocol. Finally, preparative quantities of vitellogenin nuclear RNA will be made in order to locate the eventual coding sequences within these molecules.

407. MECHANISM OF RNA METABOLISM IN ESCHERICHIA COLI

Schlessinger, D., Shen, V., Graham, M., Washington University, School of Medicine, Dept. of Microbiology & Immunology, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

The objective is to detail the mechanism by which rRNA and messenger RNA chains are degraded in E. coli. The studies are designed to characterize and assign specific RNases to these processes; and to determine how the processes are regulated. Most progress has been made to date in the characterization of the major agent of functional inactivation of lac mRNA preparations that code in vitro for the initial 'alpha-peptide' of beta-galactosidase. The agent has now been identified as a site-specific endonuclease (ms. in preparation). Studies have been started of a mutant deficient in the nuclease activity, and a screening has been devised to look for more mutants defective in the same enzyme. Its activity on other mRNA species is now being inves-

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tigated, and the precise location of its clevage of lac mRNA is being determined. Because there is an indication in vivo that the inactivating agent may only affect some mRNA's we are beginning to follow an analogous procedure to try to isolate the agents for the inactivation of other mRNA species, including that coding for the ribosomal proteins L7 and L12. Finally, we have also started to study the comparative functional inactivation and metabolism of lac mRNA and gal mRNA made from their own promoters or starting from promoters that ordinarily specify stable RNA (like rRNA or tRNA).

408. THE PRODUCTION OF IMMUNOGLOBULIN MRNA IN MYELOMA CELLS

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Immunoglobulin mRNAs, like many mRNAs in eukaryotic cells, are generated from larger nuclear RNA precursors by RNA splicing, polyadenylation and other post-transcriptional modifications. However, the molecular mechanisms of RNA splicing and the other processing events in mRNA biogenesis are not yet resolved. The ongoing studies in this proposal are directed at establishing a complete description of the transcription units and the nuclear RNA processing steps in the production of immunoglobulin heavy and light chain mRNA in MOPC 21 mouse myeloma cells. In addition to defining the active immunoglobulin genes and RNA processing pathways, we also intend to examine the DNA sequences and enzymatic mechanisms involved in RNA splicing using a mutant cell line which appears to have lost the capacity for generating heavy chain mRNA because of an alteration in nuclear RNA splicing.

409. NUCLEAR HNRNP PARTICLES IN THE MATURATION OF MRNA

LeStourgeon, W. M., Vanderbilt University, School of Arts & Sciences, Dept. of Molecular Biology, *21st Ave. S., Nashville, Tennessee*, 37232, United States of America

In mammalian cells newly synthesized premessenger RNA molecules are packaged by a unique complex of six major proteins into an array of repeating ribonucleoprotein particles (40S hnRNP particles). Considerable evidence indicates that packaging of newly synthesized RNA exists as a prerequisite to the many enzymatic RNA modifications which must occur before mature mRNA enters the cytoplasm. The ultimate experimental objective is to determine the precise composition, structure, and biochemical function of RNP particles during mRNA maturation. This objective is warranted for three principal reasons. First, research in the applicant's laboratory has shown that the protein composition of ribonucleoprotein particles differs in normal and rapidly dividing or cancerous cells. Secondly, others have observed that patients suffering from various autoimmune diseases spontaneously form antibodies which bind to and interfere with the function of RNP complexes; and thirdly, a complete knowledge of RNP particle structure and function must precede the ultimate design of therapeutic procedures. The experimental approach toward obtaining the basic information needed involves the use of highly purified native RNP's in the stepwise in vitro reconstitution of RNP particles so that the structural and functional proteins, as well as the physical means of selective RNA packaging, can be identified.

410. MESSENGER RNA METABOLISM IN MAMMALIAN CELLS

Kumar, A., Lockard, R. E., Irwin, D., George Washington University, School of Medicine & Health Sciences, Dept. of Biochemistry, *2121 Eye St. N.W., Washington, District of Columbia*, 20037, United States of America

The types of proteins and the efficiency with which they are made is often the essential determinant of phenotypic expression in cells and organisms. Changes in protein synthetic ability can be modulated either by altering the messenger RNA content or by changes in the efficiency of their translation. Since the overall objective of this research program is to understand the mechanism of mammalian cell-growth regulation, we sought to examine certain aspects of mRNA structure and function in quiescent and serum induced proliferating Balb 3T3 clone A31 cells.

The goals set for the current year were: to determine the mechanism of translational regulation in resting and serum induced 3T3 cells; secondly, to further characterize the protein associated with poly(A) regions in mRNA on the basis of its antigenic similarities; and thirdly, to focus on the structural features of a well characterized mRNA molecule in order to 'map' the protein binding sites.

411. IN SITU LOCALIZATION OF SPECIFIC BRAIN MESSENGER RNAS

Dobner, P. R., University of Massachusetts, Medical School at Worcester, School of Medicine, Dept. of Molecular Genetics & Microbiology, *55 Lake Ave. N., Worcester, Massachusetts*, 01605, United States of America

Libraries of structural gene sequences from human fetal brain, liver, heart, and muscle as well as placenta have been constructed by inserting double-stranded DNA copies of poly(A+) RNAs isolated from these tissues into the vector pBR322 and subsequent transfection of E. coli HB101. In the studies outlined here the brain library will be screened for brain-specific structural gene sequences. These clones will then be utilized to study the distribution of their corresponding RNAs by hybridization in situ to fixed preparations of sections of fetal brain mounted on glass slides. It should be possible in this way to localize the area of the brain expressing a given gene and futher to localize these transcripts in various cell-compartments (e.g nucleus and cytoplasma). These studies directly address the question of whether or not the vast diversity of sequences in mammalian brain (100,000 genes) is due to multiple functions of a complex organ each requiring its own specific set of gene products or diverse expression by each cell type. These studies should further distinguish nuclear from cytoplasmic expression. Restriction maps will be constructed to characterize the cloned sequences and their corresponding transcript(s) will be identified by northern blotting. These clones will also be used to probe natural gene structure in both somatic and germ-line tissues and could reveal novel somatic gene rearrangements. These studies should yield information on mammalian gene expression and organization and thus provide essential information pertaining to medical disorders involving abberations in gene expression.

412. THE SYNTHESIS AND PROCESSING OF THE ACTIN MRNA PRECURSOR IN DICTYOSTELIUM

McCarron, R. J., University of Massachusetts, Medical School at Worcester, School of Medicine, Dept. of Microbiology, *55 Lake Ave. N., Worcester, Massachusetts*, 01605, United States of America

Actin mRNA precursor synthesis in vitro in nuclei purified from Dictyostelium will be investigated. The structure of the actin mRNA precursor will be investigated. Once the synthesis of actin mRNA precursor has been optimized in vitro, the level of actin mRNA precursor synthesis in nuclei isolated from cells at different developmental stages will be examined. We will assess the effects of crude extracts isolated from different developmental stages on the in vitro synthesis of actin mRNA precursor. The maturation of the actin mRNA precursor will be investigated. Finally, large DNA fragments of the Dictyostelium genome will be cloned and the DNAs transcribed in vitro by purified RNA polymerase II.

413. MRNA PROCESSING IN NORMAL AND TRANSFORMED CELLS

Rottman, F. M., Horowitz, S., Sasavage, N., Camper, S., Friderici, K., Michigan State University, School of Human Medicine, Dept. of Biochemistry, *Soil Science Bldg., East Lansing, Michigan*, 48824, United States of America

Cloned sequences for bovine prolactin and growth hormone will be used to study the organization and expression of these two genes. Post-transcriptional events involved in the processing of prolactin and growth hormone mRNAs will be examined in cultured pituitary cells. The goal of this research is to define the role of mRNA capping and methylation in post-transcriptional regulation.

414. MESSENGER RNA OF NORMAL AND MALIGNANT HUMAN CELLS

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The hypothesis that cancer results from an impairment of the process of cell differentiation will be tested in tumors of the hematopoietic system. The number of different sequences (complexity) comprising the cytoplasmic polyadenylated RNA (mRNA) and the relative abundancy of these sequences will be determined. The objectives are to determine if the abundancy patterns differ among normal and malignant tissues. Cytoplasmic polyadenylated RNA, which actually does not include all the mRNA species in a cell, will be isolated, transcribed into cDNA and a cDNA library prepared. Specific cDNA clones will be selected and will be used to probe gene expression. The hybridization properties of the probe will be taken as representative of the intracellular mRNA population.

BIBLIOGRAPHIC REFERENCES: Shaw, P.A. and Saunders, G.F.: Stimulation of RNA Synthesis by Dinucleotides with Eukaryotic RNA Polymerase. FEBS Letters 106, 104-110 (1979).

415. GENE EXPRESSION IN REGENERATING AND NEOPLASTIC LIVERS

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The goals of our work are to determine the extent to which mRNA populations of adult, immature, regenerating and neoplastic rat liver may differ quantitatively and qualitatively from each other and to identify messenger RNAs (mRNA) and sets of active genes which might be specific for a certain growth pattern. We have established that a) the proportion of the non-repetitive genome transcribed is the same in normal and regenerating livers and b) that polyadenylated polysomal mRNA of regenerating and pre-neoplastic rat livers differ from the corresponding normal liver RNA only in the frequency at which some RNA sequences are represented. Qualitative differences, if they exist, are likely to be restricted to the expression of a very limited set of genes.

BIBLIOGRAPHIC REFERENCES: Scholla, C.A., Petropoulos, C.J., Becker, F.F. and Fausto, N. (1981). Alterations in polyadenylated mRNA from free and total polysomes of a rat hepatoma. Biochemistry, in press. Schultz-Ellison, G., Atryzek, V. and Fausto, N. (1981). On the existence of variant forms of gamma-glutamyl transferase in regenerating and neoplastic liver. Oncodevelopmental Biology and Medicine, in press.

416. NUCLEAR PRECURSORS OF GROWTH HORMONE AND PROLACTIN MESSENGER RNA

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Three cell lines, collectively designated GH-cells, will be employed. The GH3 rat pituitary tumor cell line synthesizes the pituitary protein hormones growth hormone (GH) and prolactin. Two variant sublines have been isolated in which the value of (GH synthesis/prolactin synthesis) is either much higher (GC cells) or much lower (GH4 cells) than in the GH3 cells. One of the proposed projects involves an investigation by the 'Northern' technique of the steady-state number, sizes and relative amounts of nuclear and cytoplasmic GH-and prolactin-specific RNA molecules in the GH-cells and the rat pituitary. The results obtained will be compared with the results of other studies in progress of the karyology of the GH-cells, and of the structure and chromosomal location in the GH-cells and the pituitary of the genes for GH and prolactin. The other project will involve kinetic experiments employing brief exposures of GH-cells to (3H)uridine. The object will be to determine the size(s) of nuclear prolactin-specific RNA molecules labeled, and whether such molecules serve as precursors of cytoplasmic prolactin mRNA. The probes to be employed in these projects will be either hybrid bacterial plasmids containing GH or prolactin complementary DNA sequences, which we presently have, or hybrid lambda bacteriophages containing the corresponding natural genes, which we are in the process of isolating and characterizing.

417. GENETIC STABILITY OF REITERATED RIBOSOMAL RNA

Petes, T. D., Zamb, T. J., University of Chicago, Pritzker School of Medicine, Dept. of Microbiology, *5801 S. Ellis Ave., Chicago, Illinois,* 60637, United States of America

I plan to characterize the molecular mechanisms which maintain homogeneity within the family of genes coding for ribosomal RNA. In each eucaryotic cell these genes are highly reiterated and share the same nucleotide sequence throughout. The processes which specify their sequence identity and preclude the toleration of sequence differences arising through mutation have yet to be determined. The yeast Saccharomyces cerevisiae now provides a model system for analysis of this problem. The recent identification of two yeast strains possessing differing rRNA genes permits genetic mapping of the rRNA gene cluster and construction of potentially unstable strains of varying heterogeneous rDNA compositions. A combined genetic and nucleotide sequence analysis of the rRNA genes from such strains will establish the existence of a sequence correction mechanism and determine the precise chromosomal arrangement of the two rRNA cluster forms, thereby defining the possible mechanisms by which sequence correction may be accomplished. The exact order of events which produce base sequence homogeneity may then be determined by rDNA analysis of the two cell division products produced immediately subsequent to a correction event. DNA recombination is thought to be central to the process of sequence correction, and therefore the effects of stimulating recombination upon the rate at which homogeneity is attained will also be tested.

418. SYNTHESIS OF MRNA IN HUMAN CELLS

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No summary has been provided to the Smithsonian Science Information Exchange.

419. MRNA FROM GLUTAMATE DEHYDROGENASE FROM EHRLICH ASCITES AND LYMPHOSARCOMA (RATS)

Hillar, M. E., Texas Southern University, School of Arts & Sciences, Dept. of Biology, *3201 Wheeler Ave., Houston, Texas,* 77004, United States of America

This is part of a broader project. A summary of this subproject is not available.

420. SYNTHESIS AND STRUCTURE OF MRNA

Jelinek, W., Rockefeller University, Viral Oncology Lab., *66th & York Ave., New York, New York,* 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

C. RIBOSOMAL RNA

421. MUTATIONAL ANALYSIS OF 5S RNA GENES IN XENOPUS

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The 5S genes of Xenopus offer a simple and well characterized system in which to study protein-nucleic acid interactions important for controlling the expression of animal genes. There are two kinds of 5S genes that are controlled differently in vivo. Both types of genes have been cloned and sequenced, and are faithfully transcribed in cell extracts. Several proteins are required for accurate transcription and one of these has been shown to bind to a region within 5S genes. I propose to address the following two important questions pertaining to the control of 5S genes: What are the specific nucleotides in 5S DNA that are involved in binding transcription factors and which are sufficient for accurate 5S RNA transcription? What are the sequences in 5S DNA which contribute to the differential expression of the two 5S DNA types? The specific aims of this proposal are: (a) To determine the contact points of transcription factors on 5S DNA. (b) To develop a general method for site-specific mutagenesis that will allow me to rapidly generate a large number of specific nucleotide substitutions in 5S DNA. (c)

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To determine the effects of mutations at different sites on the binding of transcription factors and on the expression of 5S genes in cell extracts.

422. SITE-DIRECTED MUTATIONS IN RRNA

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The study of ribosomal RNA (rRNA) is central to an understanding of protein synthesis. We now know that rRNA is more than just a backbone for holding together ribosomal proteins and is intimately involved in ribosome biogenesis and function. Unfortunately, the genetic approach to the study of 16S and 23S rRNA has been largely unsuccessful because of the inability to isolate strains containing mutations in the genes that code for rRNA. I propose to overcome this problem by utilizing several new techniques currently available at Brown University which will permit 1) the introduction of site-directed mutations into rDNA and 2) the use of rRNAs made from these mutagenized cistrons to study ribosome assembly and function.

The sequences of the 16S and 23S rRNA cistrons are known, and restricition enzyme sites are found in virtually every region of interest along the rDNA. I propose to produce deletions in vitro of defined numbers of bases starting from restriction enzyme sites in E. coli rDNA. These altered rDNAs will then be reintroduced into E. coli via transformation. The resultant recombinant DNA clones can be used to produce rRNAs in vivo and can be tested in vitro for their ability to function in reconstitution and translation assays involving ribosomal proteins, tRNAs, mRNAs, and initiation factors.

423. SECONDARY STRUCTURE IN THE 30S PRE-RRNA

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In bacterial systems such as E. coli, rRNA is transcribed as a large precursor. Processing of the large precursor involves cleavage at specific sites, trimming of ends and base modification. Several lines of evidence suggest the importance of secondary structure in the initial stages of processing. RNase III, a nuclease specific for double helices, cleaves the 30S precursor to form two smaller precursor molecules p16S and p23S along with spacer fragments.

The aim of this research is to detect secondary structure of the 30S precursor rRNA which corresponds to the initial RNase III cleavage sites using electron microscopy in conjunction with optical and enzymatic techniques. This project will include a systematic study of the effect of solution conditions on the extent of secondary structure in the precursor molecule, and to visualize these structures in the electron microscope. Specifically, by slowly increasing the temperature (or increasing formamide concentration) at a given set of ionic conditions decrease in the extent of secondary structure will be observed, which can be related to the relative stability of different regions of structure. This approach is analogous to partial denaturation mapping, a standard technique in DNA electron microscopy. Recently some preliminary experiments done in Dr. Schlessinger's laboratory indicate that micrographs can be obtained on RNA's at ambient temperature and without the use of denaturing solvents such as formamide.

424. STRUCTURE OF HYPOMETHYLATED TUMOR 5.8S RIBOSOMAL RNA

Lightfoot, D. R., Eastern Washington University, Graduate School, Dept. of Biology, *Cheney, Washington*, 99004, United States of America

5.8S rRNA occurs in the large subunit of eucaryote ribosomes and is known to form specific associations with 28S rRNA, tRNAs, other 5.8S rRNAs, ribosomal proteins, and methyl transferases. Mammalian 5.8S rRNA is deprived of one of its 2'0 methyl groups in tumors, such as rat Novikoff ascites hepatoma. Hyporibosome function or general metabolism is not known. Structural studies of 5.8S rRNA, with and without the methyl group in question (Um14), will be conducted in this project and will provide the basis for understanding the interactions with 5.8S rRNA of the molecules listed above. The role of the 2'0 methylation may be discernable. Structural techniques of nulcear magnetic resonance (NMR), partial S1 nuclease digestion, native and denaturing gel electrophoresis, hyperchromic melting, sequencing, and Sanger fingerprint mapping will be used. Control studies include structural analysis of rat, yeast, chicken, and turtle 5.8S rRNAs, and examinaton of the mechanism of dimerization of rat 5.8S rRNA.

BIBLIOGRAPHIC REFERENCES: Lightfoot, D. R., Clark, R. and Desjardins, P. R. Genomic Fragments of Turnip Yellow Mosaic Virus: Appearance During Infection. Biochem. Biophys. Res. Commun. 96, 1472-79 (1980).

425. STRUCTURE AND TRANSCRIPTION OF THE RIBOSOMAL GENE IN DROSOPHILA MELANOGASTER

Long, E. O., Dawid, I. B., Rebbert, M. L., Wood, B., Collins, M. H., Kiefer, B. I., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Developmental Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Three aspects of the structure and expression of ribosomal RNA genes in Drosophila melanogaster have been studied. First, the expression of genes interrupted by type 1 insertions in bobbed mutant flies was studied; we found that these genes are not effectively expressed in the mutant. Second, we have obtained the primary sequence of the region around the initiation of transcription of rRNA precursors in several interrupted and uninterrupted genes. Third, we have studied the structure and expression of ribosomal RNA genes interrupted by type 2 insertions.

BIBLIOGRAPHIC REFERENCES: Long, E.O. and Dawid, I.B.: Restriction analysis of spacers in ribosomal DNA of Drosophila melanogaster. Nucleic Acids Res. 7: 205-215, 1979. Long, E.O. and Dawid, I.B.: Alternative pathways in the processing of ribosomal RNA precursor in Drosophila melanogaster. J. Mol. Biol. 138: 873-878, 1980.

426. CYTOTOXICITY AND RIBOSOMAL RNA MATURATION

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The fluorinated pyrimidines are used in the treatment of cancer in man. The inhibition of the thymidylate synthetase step is a major site of action of these compounds. However, the 'activated' derivatives of fluorouracil and fluorouridine have been shown to cause the inhibition of rRNA maturation. The proposed project is designed to determine the role that the inhibition of RNA metabolism has in the cytotoxic action of these compounds. In understanding the relative importance of the effects on RNA, Novikoff hepatoma cell lines which are sensitive and resistant to fluorouracil and fluorodeoxyuridine will be used. The metabolic pathways for the 'activation' of these compounds will be studied in the two cell lines.

BIBLIOGRAPHIC REFERENCES: Cory, J. G., Okada, M., Fleischer, A. E. and Pollet, R. J., The Subunit Properties of Mammalian Ribonucleotide Reductase, Fed. Proc. 39 (1980). Cory, J. G., Stevens, A. C. and Herbst, J. S., Combination Chemotherapy Directed at the Individual Subunits of Ribonucleotide Reductase, Proc. Am. Assoc. Cancer Res. 21 (1980).

427. ENZYMATIC SPLICING OF A RIBOSOMAL RNA PRECURSOR

Cech, T. R., University of Colorado, Boulder Campus, School of Arts & Sciences, Dept. of Chemistry, *1200 University Ave., Boulder, Colorado*, 80302, United States of America

The objective of the proposed research is to characterize the RNA splicing reaction by which intervening sequences are removed from RNA molecules in eukaryotic organisms. In the amplified ribosomal RNA genes of the ciliated protozoan Tetrahymena thermophila, the 26S rRNA coding region is interrupted by a 400 base pair intervening sequence. This sequence is contained in the primary transcript but is subsequently deleted from the RNA by splicing. We have recently developed an in vitro splicing system that permits the isolation of the excised 400 nucleotide RNA fragment ('IVS RNA').

The splicing reaction will be characterized in vitro in isolated nuclei as well as in vivo to determine the nature of the reaction products and the kinetics of their synthesis and degradation. Hybridization of nuclear RNA with recombinant DNA containing the intervening sequence will be used to isolate the

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unspliced pre-rRNA. This material will provide the basis for an assay to detect splicing activity, which should allow purification of the enzyme(s) involved. The enzyme(s) may require an rRNA-protein complex as a substrate, in which case nascent ribosomal particles will be isolated. The subunit structure, cofactor requirements, and specificity of each enzyme will be investigated. Temperature-sensitive mutants of Tetrahymena that are known to accumulate pre-rRNA at the nonpermissive temperature will be screened for splicing activity.

428. GENE REGULATION IN DIPLOID AND HYBRID MAMMALIAN CELLS

Miller, O. J., Tantravahi, R., Columbia University, College of Physicians & Surgeons, Dept. of Human Genetics & Development, *630 W. 168th St., New York, New York*, 10032, United States of America

The object of this study is to clarify the mechanism of regulation of ribosomal RNA (rRNA) synthesis and nucleolus organizer (NO) activity in interspecific hybrids in which there is species-specific suppression of these two activities. The role of DNA methylation in the suppression of rRNA gene transcription will be investigated by immunological and restriction enzyme methods. The suppression of rRNA synthesis and NO activity will be correlated with the loss of specific human or rodent chromosomes in mouse-human and rat-human hybrid cells that selectively lose human or rodent chromosomes. Reactivation of inactive NO regions will be attempted by back hybridization to one of the parental cell types containing distinguishable NOR chromosomes. Suppression will be looked for in heterokaryons, in diploid x diploid somatic cell hybrids, and in hybrid animals.

429. RNA METHYLATION - A PARADOX IN NORMAL AND CANCER CELLS

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The sequence of 5.8S ribosomal RNA has recently been established in several mammalian species including man. One position in the sequence contains a partial 2'-O-methylation which occurs in lower yields in tumor cells. The percent -UmG- was found to vary from 17 to 72% when examined in a variety of tumor and normal tissues, yet another methylated position, -GmC-, remained constant at 100% (1 molar yield). The role of methylation is not yet clear; however; it appears to be important for normal processing of ribosomal RNA. Recent work has also shown that transfer RNA methylation is reduced in tumor cells (both cytoplasmic and mitochondrial tRNA) suggesting a general phenomenon of undermethylation in cancer cells. In contrast to the in vivo undermethylation of RNA, the methylase activity for both base methylation in tRNA and ribose methylation in RNA appears to be elevated when measured in vitro. Tumor cells may produce excess enzyme in an attempt to compensate for the low methylation levels, but because of inhibition or lack of substrate, S-adenosyl-methionine (SAM), this has no effect.

Through a study of the in vivo and in vitro methylation of ribosomal RNA at specific sequences, the paradox of low RNA methylation and elevated methylase activities in tumor cells may be explained. Primary cultures of rat kidney cells, the normal rat kidney (NRK) cell line, NRK cells transformed by wild-type mouse sarcoma virus (MSV), or a temperature-sensitive (ts) transformation mutant of MSV, and a ts mutant of baby hamster kidney cells unable to process rRNA, will be used to examine the effect of culture conditions such as growth rate, passage number, and methionine concentration on methylation. The levels of SAM, S-adenosylhomocysteine, and methionine transport will be monitored and compared to ribosomal RNA methylation and methylase activities in these cells. An enzyme assay for the methylation of the specific -UmG-position in 5.8S rRNA will be developed and will be useful in attempts to purify the enzyme and to determine the part of the sequence and structure of 5.8S rRNA necessary for enzyme recognition.

430. REGULATION OF GROWTH HORMONE MESSENGER RNA

Bancroft, F. C., Szabo, P., Sloan Kettering Inst. for Cancer Research, Laboratory of Cellular Gene Expression & Regulation, *410 E. 68th St., New York, New York*, 10021, United States of America

The research involves two major projects. The aim of one project is to determine the number, gross structure, and chromosomal locations in normal rat cells and in rat pituitary tumor cells (GH-cells) of the genes for the pituitary hormones, prolactin, growth hormone, and the alpha and beta subunits of thyrotropin. We are currently screening a rat genomic library for recombinant lambda bacteriophages containing these genes. These hybrid bacteriophages will then be employed as probes for chromosomal localization by in situ hybridization. The aim of the other project is to determine the primary site(s) at which thyroid and glucocorticoid hormones act to increase growth hormone synthesis by GH-cells, and to investigate the role of insulin in the synergistic regulation by these two types of hormones of growth hormone gene expression. A recombinant bacterial plasmid containing a DNA sequence coding for rat growth hormone which we have prepared will be employed as a hybridization probe in these studies to follow nuclear and cytoplasmic growth hormone-specific RNA sequences during hormonal induction.

431. ORGANIZATION AND EXPRESSION OF RIBOSOMAL PROTEIN GENES

Monk, R. J., Inst. for Cancer Research, Fox Chase Cancer Center, *7701 Burholme Ave., Philadelphia, Pennsylvania*, 19111, United States of America

The objective of this research is the understanding of the structure and regulation of ribosomal protein genes (r-protein) in eukaryotic cells. The problem will be approached at the molecular level by using recombinant DNA techniques. R-protein cDNA clones will be isolated and then used to isolate r-protein genes from an existing mouse clone library. The cDNA clones and the genomic clones will then be mapped in detail and then used to study the following: 1) gene number and the order of individual small and large subunit r-protein genes, 2) the determination of the lifetime of particular r-protein mRNAs under normal physiological conditions, 3) the size of the nuclear RNA and cytoplasmic RNA molecules containing r-protein mRNA sequences and verification that the large nuclear molecules are r-protein mRNA precursors, 4) the relative abundance of individual r-protein sequences in the nucleus and cytoplasm and rates of transcription, 5) processing pathways of particular r-protein precursor RNA. These studies will result in the determination of the structure, organization, and understanding of the coordinate regulation of as many as 70 different r-protein genes and the results compared to those obtained with other gene systems currently being studied in other laboratories.

432. STUDY OF RNA PROMOTER SITES

Krueger, J. A., University of Wisconsin, Madison Campus, Inst. for Enzyme Research, Dept. of Biochemistry, *750 University Ave., Madison, Wisconsin*, 53706, United States of America

The objective of the project is to identify the promoter(s) of rRNA operons in Escherichia coli and the precise start site(s) of rRNA transcription in vivo. 5'-terminal portions of rRNA operon will be isolated with their 5'-end triphosphate intact; their nucleotide sequence will be determined. Transducing phages carrying rRNA operons will be used to isolate mutants, either deletion or point mutants. One of the approaches to be used is to fuse an rRNA promoter region to the lac operon and use various selection methods employed in the isolation of lac promoter mutants.

D. TRANSFER RNA

433. ALTERATIONS IN THE STRUCTURE AND FUNCTION OF TRANSFER RNA DURING FRIEND CELL DIFFERENTIATION

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OBJECTIVE: We are examining the roles played by altered tRNAs in cell transformation by studying the structure and function of tRNAs which become altered during the differentiation of the Friend cell. This cell is a murine tumor tissue culture cell which stops dividing either when a high cell density is reached or when the cell is chemically induced to undergo erythyroid differentiation.

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APPROACH AND PROGRESS: We have found that when Friend cells stop dividing by either of these pathways, certain tRNAs become altered as determined by their chromatographic properties using reverse phase chromatography. The changes occurring in the phenylalanine and lysine tRNA families appear similar to those which have been found in other tumor-non tumor systems. We are studying these changes in more detail by purifying the lysine and phenylalanine isoacceptors and analyzing their altered structure and function to determine what role these altered tRNAs might play in cell transformation. For example, the presence of one lysine tRNa isoacceptor, lys tRNA4, has been shown to be correlated with the ability of a cell to divide, but recently we have found that the concentration of this tRNA species can remain high in non-dividing Friend cells which produce virus, and we are currently testing whether there exists a relationship between viral gene expression and the presence of this tRNA in mammalian cells. We are also testing to see if this lysine tRNA species, as well as tumor-specific phe-tRNA1, are altered in their ability to support protein synthesis.

434. THE STRUCTURE AND FUNCTION OF SUPPRESSOR TRNAS INVOLVED IN VIRAL INFECTION

Rich, A., Auron, P. E., Massachusetts Inst. of Technology, School of Science, Dept. of Biology, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

The proposed research will be aimed at elucidating the structure of a suppressor tRNA molecule isolated from reticulocyte extracts by Geller and Rich (manuscript submitted for publication) in order to understand its mechanism of action. Additionally, the hypothesis that there exists a relationship between viral host specificity and tRNA suppression will be investigated using readthrough of know termination codons as an assay of suppressor activity in various tissues.

Several investigators have demonstrated the importance of termination suppression to produce longer-than-normal 'readthrough' protein products required for the viability of some prokaryotic and eukaryotic viruses. The isolation, by Geller and Rich, of a UGA suppressor in rabbit reticulocytes supports the hypothesis that such tRNAS exist and could be utilized by infecting viruses.

The goals of this project will be to purify this reticulocyte UGA suppressor and fully characterize it by determining such information as methylation pattern, nucleotide sequence, and structural conformation using already established techniques. The occurrence of suppressor tRNA molecules in various tissues will be determined using an in vitro translation system, to which has been added messages with known termination codons, and assaying for readthrough proteins produced in the presence of tRNA preparations from various tissue extracts. The hypothesis that a relationship between infection specificity and suppressor tRNA can then be tested by determining if any correlation exists between tRNA suppressors in tissues and the specificity of viral infection.

435. TRANSCRIPTION OF T-DNA IN CROWN GALL TUMORS

Grula, M., Washington University, School of Arts & Sciences, Dept. of Biology, *4559 Scott Ave., St. Louis, Missouri,* 63130, United States of America

The goal of this work is to understand in more detail the transcription of T-DNA (the foreign DNA of bacterial origin) in crown gall tumors. The proposed studies will examine the reliance of T-DNA transcription on plant RNA polymerase enzymes, the relationship between the initiation of plant and T-DNA transcription and the T-DNA transcriptional products.

The plant RNA polymerase that transcribes T-DNA will be identified by using an inhibitor of eucaryotic RNA polymerases and once this enzyme is determined, it will be used in filter binding studies to locate RNA polymerase binding sites on restriction enzyme fragments of cloned T-DNA. The binding sites, or putative promoter regions, will be sequenced to determine homologies to known procaryotic or suspected eucaryotic promoters. T-DNA transcripts will be isolated from polysomes of tumors and these transcripts will be placed on a physical map of T-DNA to determine exactly which portions of the T-DNA are transcribed and activiely translated in tumors.

436. TRANSCRIPTIONAL ROLE OF THE INSERTION IN THE XENOPUS TRNA TYR GENE

Hipskind, R. A., Universite de Geneve, Faculte de Medecine, Dept. de Microbiologie, *40 Blvd. de la Cluse, 1211 Geneva 4, Switzerland*

The Xenopus tyrosine tRNA gene is the first vertebrate tRNA gene reported that contains an insertion. This gene will be subcloned alone and will be transcribed in vitro in a post-mitochondrial supernatant prepared from Xenopus culture cells or from mature Xenopus oocytes. The precursors and mature tRNA product will be characterized by two dimensional fingerprint analysis and the initiation site will be mapped by analysis of the 5' termini of the in vitro transcripts. An Eco RI site will then be placed within the insertion sequence. Deletions of DNA will be created in the insert from this RI site and their effect of transcription in vitro assayed. Likewise, the effect of adding specific and non-specific DNA sequence to the insert will be analyzed. From these results the role of the insertion sequence in the control of transcription and/or in regulating maturation of tRNA precursors can be determined. To identify the specific sequences and bases involved in such regulatory activities the sequence within the insert will be mutagenized and the mutant clones affecting transcription will be sequenced.

437. TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL CONTROL OF TRNA SYNTHESIS IN YEAST

Ciejek, E. M., University of California, San Diego Campus, Graduate School, Dept. of Chemistry, *P.O. Box 109, La Jolla, California,* 92037, United States of America

OBJECTIVES: This research will investigate the transcription of certain yeast tRNAs and the removal of intervening sequences from the primary transcripts. Efforts will be made to define (1) transcriptional start and stop sites, (2) if and how excision and splicing is controlled (3) the physiological significance of this control and (4) the relationship between transcription and post-transcriptional processing. These investigations will help determine the specificity of RNA polymerase III, which has been shown to be responsible for 5S and tRNA sysnthesis in yeast and higher eucaryotes. These studies will also elucidate the role or lack thereof of intervening sequences in the regulation of transcription and post-transcriptional processing.

METHOD OF APPROACH: One of the genes for tRNA-tyr, corresponding to the genetic locus SUP 4, has been cloned and sequenced. There is an intervening sequence 14 nucleotides long in the gene which is not found in the mature tRNA. This gene will be altered in vitro by deletions, additions or base alterations. Alterations of the gene will include (1) deleting or altering leader or promoter regions and termination regions (2) deletions of or additions to the intervening sequence and (3) deletions or alterations of splicing junctions. The specific sites of these mutations will be defined through a combination of restriction enzyme mapping and direct nucleotide sequence determination. First, the efficiency of transcription of the altered genes will be measured in vitro in the Xenopus germinal vesicle extract system. Altered genes which are successfully transcribed will be used to obtain altered precursor tRNAs, which may be isolated by transformation of the ts 136 mutant, and used to investigate the specificity of purified splicing enzyme. Then, the efficiency of processing of the transcripts will be determined by using the altered tRNA genes to transform yeast strains containing nonsense mutations.

438. TRNA, PROTEIN-NUCLEIC ACID INTERACTIONS

Schulman, L. H., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Dept. of Developmental Biology & Cancer, *1300 Morris Park Ave., Bronx, New York,* 10461, United States of America

This is part of a broader project. A summary of this subproject is not available.

439. STUDIES ON THE MECHANISM OF ACTION OF AN RNA SPLICING ENZYME

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Most eukaryotic genes contain intervening sequence stretches of DNA which interrupt the coding sequence of a gene. These intervening sequences are removed from RNA

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transcripts of the gene by a process known as RNA splicing. We are studying the mechanism of RNA splicing in the yeast tRNA system. We have detected an activity which removes the intervening sequence from a tRNA precursor to generate the mature tRNA and the excised intervening sequence. We now know that the reaction can be divided into two steps. In the first step an endonuclease cleaves the RNA, releasing the intervening sequence as a linear RNA molecule with 5'-hydroxyl and 3'-phosphorylated termini. In the second step, which requires ATP, the two half molecules are joined together by an RNA ligase. The RNA ligase has a different specificity from any ligase previously described. It forms a phosphodiester bond by joining a 3'-phosphate to a 5'-hydroxyl. DNA ligases and the T4 RNA ligase do the opposite, joining 5'-phosphates to t 3'-hydroxyls. The usual specificity of the opposite, joining 5'-phosphates to 3'-hydroxyls. The usual specificity of the tRNA splicing ligase has led us to speculate that this may be a common feature at the heart of all RNA splicing but this of course remains to be proven. The two activities, the endonuclease and the ligase are separated in the first step of our purification scheme but when reconstituted function to carry out the entire splicing reaction. We have found that the endonuclease is associated with membrane in yeast while the ligase is a soluble enzyme. These findings raise the intriguing possibility that the endonuclease may be an integral part of the nuclear membrane, possibly of the pore. We propose to purify both of these activities to homogeneity, to study their mechanisms of action and to localize the activities in the cell.

440. CHARACTERIZATION OF YEAST TRNA SPLICING

Schwartz, R., University of California, San Diego Campus, Graduate School, Dept. of Chemistry, *P.O. Box 109, La Jolla, California*, 92037, United States of America

I propose to study the requirements of the splicing activity involved in the processing of yeast tRNA precursors. Specifically modified tRNA genes will be introduced into yeast by transformation of hybrid bacterial plasmids containing the modified tRNA genes. Modifications will be introduced into the tRNA genes by priming DNA synthesis on the wild-type plasmid with an oligonucleotide complementary to the desired mutant sequence. Subsequent segregation of the heteroduplex products after transformation into E. coli will produce the desired mutant plasmid. Deletions in the tRNA genes will be introduced by Exo III digestion at specific restriction sites followed by S1 nuclease digestion and then ligation with T4 ligase.

Genes coding for nonsense suppressor tRNAs will be used to allow a simple biological assay for expression. tRNA genes rendered incapable of nonsense suppression by modification will be further analyzed by examination of their transcription products by two-dimensional gel electrophoresis, ribonuclease fingerprinting and RNA sequencing.

I shall also attempt to assay the ligation activity of yeast tRNA splicing with simple artificial substrates. If this proves successful, assays will be attempted in other systems with the hope of assaying a ligation activity associated with mRNA processing.

441. THIOLATED TRNAS IN RAT LIVER AND MORRIS HEPATOMAS

Wong, T., University of Chicago, Pritzker School of Medicine, Dept. of Pathology, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

This project is concerned with the study of thionucleotides in tRNA. The best known function of thionucleotides involves the 2-thiouridine derivatives found in the anticodon of a number of prokaryotic and eukaryotic tRNAs. Such thionucleotides have been shown to be essential to precise codon recognition. At present, three mammalian tRNAs are known to contain 2-thiouridine derivatives in the anticodon; these include one isoaccepting species each of tRNAGlu, tRNAGln, and tRNALys.

In previous studies, we isolated a tRNA sulfurtransferase system from the cytosol of the liver of Buffalo rats. This enzyme is capable of thiolating tRNA in vitro by catalyzing the transfer of labeled sulfur from (35S)Beta-mercaptopyruvate to tRNA, forming thionucleotides. The activity of this enzyme is markedly decreased in Morris hepatomas compared to normal rat liver and is due to the presence of an inhibitor in the tumor cytosol which suppresses the transsulfuration reaction. To determine whether this inhibitor of the in vitro transsulfuration reaction has a significance in vivo in terms of suppressing tRNA thiolation and causing derangements of tRNA function, we propose the following studies: 1) The three species of tRNAs known to contain a 2-thiouridine derivative in the anticodon will be isolated from normal rat liver and sequenced by post-labeling methods with 32P. 2) The three corresponding tRNAs from the fast-growing Morris hepatoma 9A will be isolated and sequenced similarly. 3) If the tumor tRNAs show deficient thiolation in the anticodon, their coding properties will be studied and compared with those of the corresponding normal liver tRNAs. 4) The pair of normal and tumor tRNAs showing the most divergence in primary structures will be examined by high-resolution NMR spectroscopy to determine how abnormalities in the primary structure of the tumor tRNA can affect its three-dimensional structure and in turn its biological function.

442. SEQUENCING THE GENE FOR GLYCOL-TRNA SYNTHETASE

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Aminoacyl-tRNA synthetases are common to all living organisms and, as a class, are among the most important enzymes in living systems. Very little information is available at the molecular level about the primary structure of the synthetases. Also, despite extensive physiological data, very little is known at the molecular level about controls of aminoacyl-TRNA synthetase formation. Therefore, the purpose of this study is to isolate and sequence the Gly-tRNA synthetase structural gene and the region preceding the structural gene. This gene has been cloned on a hybrid ColEl plasmid carried by an E. coli strain. We will attempt to isolate the glyS locus onto a much smaller DNA fragment contained on a pBR322 plasmid, using standard shotgun cloning techniques and a temperature sensitive mutant. After recloning the glyS locus, the gene will be located on a still smaller piece of DNA by testing fragments of the pBR322 insert for a strong promoter. Finally, once such a fragment has been isolated, its entire DNA sequence will be obtained using the method of Maxam and Gilbert (PNAS (1977) 72, 560-564).

443. SEQUENCE MAINTENANCE IN DISPERSED MULTIGENE FAMILIES

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It is clear that the tRNA in yeast and some other dispersed multigene families in eukaryotes are maintained as homogeneous families by a novel mechanism. I have hypothesized that homogeneous families may result from recombination among family genes on nonhomologous chromosomes. The known arrangement of yeast tRNA intervening sequences is compatible with a role in limiting such recombination events to members of the same family.

The yeast tRNA Tyr gene family is well-defined and provides an ideal system for evaluating the evolutionary pressures which influence dispersed multigene families. Three specific questions will be addressed: 1) Is there strong functonal selection in the tRNA Tyr family for maintenance of wild-type sequence and gene number? tRNA Tyr genes will be sequentially deleted from a yeast strain and the minimum number of essential tRNA Tyr genes will be determined; strains carrying deletions will be observed for evidence of expanding gene number. 2) Why is the tRNA Tyr intervening sequence conserved? The intervening sequence will be removed from a suppressor tRNA gene. The suppressor activity and capacity of this gene for recombination with other tRNA Tyr genes will be measured. 3) Do tRNA Tyr genes on nonhomologous chromosomes recombine? A small deletion will be introduced into a suppressor tRNA Tyr gene and rescue by combination with other tRNA Tyr genes on nonhomologous chromosomes will be scored.

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E. OTHER STUDIES OF RNA BIOCHEMISTRY

444. STUDIES ON ELECTROPHORETIC TECHNIQUES FOR PROTEIN, RNA, AND DNA

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A new method for estimation of chain lengths of ribonucleic acids is under development. The method involves partial heat degradation of the RNA under study and measurements of the remaining and degraded molecules. From similar measurements on reference RNAs, the chain lengths may be deduced. In addition, computer simulation permits computation of the relation between chain length and electrophoretic mobility for use in electrophoretic analysis of polydisperse samples. Heterogenous nuclear RNA from HeLa cells has been studied by these techniques. Purification of RNA and DNA by means of conventional and electrophoretic techniques has been achieved, principally through the use of apparatus designed elsewhere. The purified material facilitates study of the properties of these molecules.

BIBLIOGRAPHIC REFERENCES: McIntosh, P. R., Peacock, A. C., and Gelboin, H.V.: The induction of liver microsomal proteins by 3-methylcholanthrene in rats of different age, sex and strain. Biochim. Biophys. Acta 627: 290-300, 1980.

445. CONTROL OF PROTEIN SYNTHESIS BY DOUBLE-STRANDED RNA

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The biological significance of double-stranded RNA (dsRNA) is reflected in the increasing evidence which indicates that dsRNA exerts regulatory functions in a wide range of eukaryotic biological systems including normal and tumor tissues, cultured cells, and cell-free extracts. Some of these effects include (1) the induction of interferon, (2) a cytotoxic effect on cells, (3) the suppression of tumor growth and development, (4) an enhancement of the immune response, and (5) the potentiation of inhibitory effects in cell-free extracts. One of the most distinctive effects of dsRNA is its capacity to promote an antiviral state in tissues and cells similar to that achieved by interferon. Significantly, treatment of cells with interferon causes the induction of several dsRNA-regulated components, two of which are potent inhibitors of protein synthesis. These are (a) a cAMP-independent protein kinase which phosphorylates the alpha-subunit of eIF-2, the protein chain initiation factor which activates initiator methionyl-tRNA; and (b) a (2-5A)n synthetase which polymerizes ATP into short oligonucleotides linked by (2'-5')-phosphodiester bonds of the form (pppA(2')p(5')A (2')p(5')A); these oligomers serve as activators of endonuclease activity.

In addition to interferon-treated cells, the two dsRNA-regulated activities are found in normal and tumorigenic cells, and in reticulocyte lysates. The physiological roles of these functions are not clear. The induction of these two activities in interferon cells suggests that they are involved in the development of the antiviral state. The presence of the two activities in normal cells suggests that they are also involved in normal physiological processes.

The primary objective of the proposed study is to determine the mechanisms by which dsRNA regulates macromolecular mechanisms with emphasis on the control of translation. The specific research goals will focus on the (a) isolation and characterization of the two dsRNA-regulated activities; (b) examination of their mechanisms of activation and expression; and (c) their physiological functions in normal, tumorigenic and virus-infected cells.

BIBLIOGRAPHIC REFERENCES: Daniel Levin, Vivian Ernst, and Irving M. London. Effects of the catalytic subunit of cAMP-dependent protein kinase (Type II) from reticulocytes and bovine heart muscle on protein phosphorylation and protein synthesis in reticulocyte lysates. Journal of Biological Chemistry (1979) 252, 7935-7941. V. Ernst and D. Levin. In situ phosphorylation of eIF-2 in reticulocyte lysates. Fed. Proc. 38:2913, 1979.

446. RNA METABOLISM IN DEVELOPMENT AND HORMONAL REGULATION

Key, J. L., University of Georgia, Franklin College of Arts & Sciences, Dept. of Biochemistry, *Athens, Georgia*, 30601, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

447. 'TUMOR IMMUNE' RNA - A BIOCHEMICAL CHARACTERIZATION

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This proposal is an interdisciplinary approach to a biochemical assessment, isolation, and characterization of immunobiologically active RNA molecules able to transfer tumor specific and/or non-tumor specific sensitivity to lymphoid cells. Our objectives are the following: 1) To isolate and fractionate RNA from lymphoid tissues of syngeneic strain-2 guinea pigs immunized against line-1 or line-10 tumors; or other non-tumor antigens, KLH or ARSNAT. 2) Each RNA fraction will be assessed for its ability to tranfer tumor or other antigenic specific sensitivities to guinea pig peritoneal exudate cells in vitro; utilizing the agarose droplet cell-migration-inhibition correlate of delayed type hypersensitivity and the thymidine incorporation assay. 3) Fractions of RNA capable of converting lymphoid cells will be purified by Oligo-dT-cellulose chromatography and polyacrylamide gel electrophoresis. 4) The RNA fraction which is biologically active will be used as templates for reverse transcriptase. The cDNA product will be cloned in plasmid pBR322. RNA from clones containing hybrid plasmid DNA will be tested for biological activity. 5) DNA from the active clones will be further characterized biochemically. 6) Fractions of RNA capable of converting lymphoid cells will be further characterized as to their homogeneity and/or heterogeneity of the biologically active RNA species and the number of RNA molecular species found in the active extracts. 7) To assess the molecular weight and class of RNA to which the active species belongs. 8) Biologically active RNA species will be assessed for the presence of 'capped' structures in the poly-A containing Fraction II, possessing immunobiological activity. 9) The base composition and sequence of the biologically active RNA fraction(s) will be determined. 10) To assess at each step of the purification stages, the biological activity of the isolated RNA molecules. 11) To compare the nucleotide sequences of active RNA species between different lymphoid tissues of the same animal; and from different animals immunized against tumor or chemically defined antigens.

448. ISOLATION AND CHARACTERIZATION OF DNA-INDEPENDENT RNA POLYMERASE II FROM ROOSTER LIVER

Mullinix, K. P., Kastern, W. H., Eldridge, J. D., Sprague, J. A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cellular Regulation Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

We have developed a procedure for isolating DNA-dependent RNA polymerase from rooster liver nuclei that involves treatment of nuclei with pancreatic DNAse I to partially solubilize chromatin. The enzyme thus obtained is completely dependent on the addition of exogenous DNA for activity. We have developed a technique for identifying RNA polymerase II in nuclear lysates by binding 3H alpha-amanitin to the polymerase and subsequently subjecting the proteins to electrophorese in polyacrylimide gels under non-denaturing conditions. By this method we have identified two alpha-amanitin binding proteins, tentatively identified as two forms of RNA polymerase II. Unpurified preparations of RNA polymerase II prepared in this manner had the ability to transcribe SV40 DNA asymmetrically in the region of the origin of replication.

BIBLIOGRAPHIC REFERENCES: Kastern, W.H., Eldridge, J.D., and Mullinix, K.P.: DNAse I digestion of chromatin from avian liver nuclei liberates DNA-dependent RNA polymerase II: Isolation of RNA polymerase II by the DNAse I-Sepharose digestion. J. Biol. Chem. 254: 7368 (1979). Kastern,

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W.H., Stanchfield, J.E., Eldridge, J.D., and Mullinix, K.P.: In vitro transcription of SV40 DNA by nuclear extracts form avian liver. J. Cell Biol. 83: 406a (1979).

449. GENETICS AND PHYSIOLOGY OF E. COLI RNA POLYMERASE

Oeschger, M. P., Georgetown University, School of Medicine, Dept. of Microbiology, *3800 Reservoir Rd. N.W., Washington, District of Columbia*, 20007, United States of America

This grant involves continuation of support provided under grant BMS75-21305 for Dr. Oeschger's research program. Studies on the genetics and physiology of E. coli RNA polymerase are being extended. Dr. Oeschger is using his temperature sensitive suppressor strains carrying amber mutations in rpoB (the B subunit gene for the polymerase) to study the effects of the level of polymerase on the expression of polymerase and ribosomal genes, to titrate the level of functional polymerase in cells growing under a variety of nutritional conditions, and to isolate additional mutants in which the BB' operon is not regulated. In addition he is using his ts suppressor strains to isolate and study amber mutations in rpoA and rpoC (the alpha and B' subunit genes). He is complementing these studies with the investigation of a number of other ts mutants which he has isolated. This group includes not only mutants which are ts for RNA synthesis. In collaboration with Patrick Dennis, he is determining whether the regulation of polymerase gene expression in these (as well as the amber mutants) is transcriptional or translational. Finally he plans to characterize the mutations which are responsible for the super-suppression in two of his ts suppressor strains. These studies should provide fundamental information about the regulation of the expression of the polymerase genes, control of polymerase activity within the cell, and ribosomal and RNA gene expression.

450. STUDIES ON THE ROLE OF RNA POLYMERASE IN ATTENUATION

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Attenuation is a phenomenon found in certain prokaryotic biosynthetic operons in which translation modulates transcription. A model for the mechanism of attenuation proposes that the leader mRNA's ability to form stem and loop secondary structures in a sequential and transient manner as the leader transcript is synthesized by RNA polymerase and translated by a ribosome determines the fate of the polymerase: whether to continue RNA synthesis (readthrough) into the structural genes of the operon, or to terminate RNA synthesis at the attenuator. It is unclear how the translating ribosome makes its position known to the transcribing RNA polymerase. I propose to discover which portions of the transcript interact with which subunits of polymerase in termination complex at the attenuator. I will determine which segments of the transcript interact with which subunits of RNA polymerase by the ability of the polymerase to protect these regions from RNase and DNase digestion, and analyze which segments of the template and transcript are base paired and single stranded. I will attempt to crosslink RNA polymerase to the attenuator termination complex, and select for polymerase mutants with altered transcription termination at the tryptophan attenuator.

451. FUNCTION OF RNA POLYMERASE DURING TRANSCRIPTION OF A SPECIFIC GENE SET

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The specific goal of the project is to isolate and characterize the factors responsible for selective RNA transcription in eucaryotes. The system to be utilized is the heat shock response exhibited by Drosophila melanogaster cells. This response is induced by mild heat shock (37 degrees C for 20-40 minutes) of whole larvae, isolated salivary glands or cultured cells. In the giant polytene nuclei of the salivary glands, the heat shock response is characterized by the simultaneous appearance of nine new chromosomal puffs and the regression of any previously existing puffs. In tissue culture cells the response is monitored by the appearance of a new pattern of RNA synthesis and the shutdown of normal RNA synthesis. This suggests that heat shock evokes the coordinate turn-on of a small number of genes and the turnoff of previously active genes. In this study we hope to isolate and characterize the factors which cause the selective transcription of this small gene set. The actual isolation of selectivity factors will involve the use of mercurated amanitin. Chromatin from heat shocked D. malanogaster cells will be isolated and digested with pancreatic DNase, micrococcal nuclease or other restriction nucleases. This will yield different length chromatin fragments containing RNA polymerase that can be reacted with mercurated amanitin and then passed through a sulfhydryl column. The column specifically binds mercury and non-specific material is removed by washing with buffer. This method will isolate transcription chromatin complexes which should contain the selectivity factors. Comparison between control and heat shocked cells after the isolated polymerase-chromatin fragments are subjected to SDS gel electrophoresis will indicate which proteins are generally associated with chromatin and which are unique to the heat shock chromatin. The chromatin complex can be dissociated with high salt and the proteins fractionated by molecular sieve chromatography. The proteins can then be characterized by their DNA-binding and RNA polymerase affinity. The appropriate proteins can be tested for their selectivity by using them in in vitro RNA polymerase assays utilizing naked DNA or non-heat shocked chromatin.

452. STUDIES ON THE UNWINDING OF DNA BY RNA POLYMERASE

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The binding of E.coli RNA polymerase to a DNA promoter site involves a slight unwinding of the DNA helix. It has recently been shown that the extent of unwinding is influenced by temperature and ionic strength.

Previous experiments have measured the average extent of unwinding of several polymerase molecules bound to a single DNA molecule. The proposed project is an attempt to determine the effects of temperature and ionic strength on the unwinding caused by binding at a single promoter site. The experiments involve measurement of the average unwinding angle of polymerases bound to two almost identical templates to be constructed in vitro. One template will consist of a long piece of DNA known to contain no promoter sites. The other will contain this sequence and a single short promoter region. Comparison of the average unwinding angles of polymerases bound to these templates will indicate the unwinding angle of polymerase bound at the strong promoter. Hopefully, it will be possible to correlate the unwinding angle with the transcriptional activity of the strong promoter.

453. RNA POLYMERASE INITIATION FACTOR FROM LEUKEMIC CELLS

Chuang, R. Y., Chuang, L. F., University of California, Davis Campus, California Primate Research Center, *Davis, California*, 95616, United States of America

OBJECTIVE: The research is to study the RNA polymerase initiation factor we previously isolated from chicken leukemic x cells and isolate the same factor from other leukaryotes.

APPROACH: Experiments include purification of the factor, determination of its subunit composition and its activity on fractionated chromatin, study of the mechanism by which it stimulates RNA chain initiation, and isolation of factor-specific antibody. Determination of the factor activity in chicken myeloblastosis virus non-producer cells, human leukemic-cells and normal human leukocytes will reveal whether this factor is leukemic-specific or it is required for all normal eukaryotic RNA synthesis initiation.

PROGRESS: 6-Mercaptopurine (6-MP) is extensively metabolized to phosphorylated and/or methylated metabolites in leukemic patients undergoing chemotherapy. We found that, in vitro, 6-MP ribonucleoside triphosphate (6-MPTP) can inhibit DNA-dependent RNA polymerase isolated from Escherichia coli and chicken leukemic cells competitively. This inhibition causes a decrease in RNA product size. The 6-MP monophosphate and 6-MP riboside exhibited little or none of this inhibi-

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tory activity. Data suggest that this inhibition may contribute to 6-MP cytotoxic effects.

454. IDENTIFICATION OF RNA SEQUENCES FOR USE AS PREDICTIVE MARKERS IN THE TREATMENT OF HUMAN BREAST CANCER

Craig, R. K., Hall, L., Courtauld Inst. of Biochemistry, *London, England, United Kingdom*

OBJECTIVE: To identify RNA species in human breast tissue and human mammary derived cell lines, which may prove useful as markers of clinical relevance.

APPROACH: We have constructed a library of recombinant plasmids containing cDNA sequences synthesized from poly(A)-containing RNA isolated from normal human lactating mammary tissue (retention cyst). We intend to select and characterize from this library sequences which are abundant or moderately abundant in breast tumor tissue, but of lesser abundance in normal breast tissue. Where quantitative differences are identified we will determine whether or not these sequences are likely to be of clinical value as predictive markers in the treatment of breast cancer.

455. ROLE OF OOCYTE PROTEINS AND RNAS IN DEVELOPMENT

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No summary has been provided to the Smithsonian Science Information Exchange.

456. RIBONUCLEIC ACID AND PROTEIN CHANGES IN MALE AND FEMALE PRONUCLEI AND ZYGOTE NUCLEUS OF THE SEA URCHIN, STRONGYLOCENTROTUS PURPURATUS

Irving, C. C., University of Tennessee, Center for the Health Sciences, School of Medicine, Dept. of Urology, *62 S. Dunlap St., Memphis, Tennessee*, 38103, United States of America

The objective of this research is to define the biochemical changes that occur during the activation of the paternal chromatin, i.e., the activation which occurs when the sperm nucleus is incorporated into the egg cytoplasm at fertilization. This objective will be met by answering the following two questions: First, does the male pronucleus synthesize RNAs, and if so, what types? Second, what are the changes in the nuclear proteins during activation of the paternal chromatin?

The experimental approach is to fertilize sea urchin eggs in the presence of RNA or protein precursors. After varying amounts of time the zygotes are homogenized, and the pronuclei extracted on a discontinuous sucrose gradient. The zygote nucleus will be analyzed at this time without further manipulation. The pronuclei are layered on a linear sucrose gradient and the male and female pronuclei separated due to difference in size. RNA will be extracted in hot phenol-SDS and analyzed on sucrose gradients. Proteins will be extracted in phenol and analyzed on acrylamide gels. Protein changes will be monitored by observing the changes in banding patterns on gels as the paternal chromatin is activated, i.e., the proteins present in the sperm nucleus will be compared to those present in the male pronucleus and the zygote nucleus.

IV. PURINE AND PYRIMIDINE METABOLISM

457. PURINE TRANSPORT BY MEMBRANE VESICLES OF CANCER CELL LINES

Hochstadt, J., Prasad, R., New York Medical College, Westchester Campus, School of Medicine, Dept. of Microbiology, *Valhalla, New York*, 10595, United States of America

The vesicle transport system developed in previous years to assess the role of transport in cancer is extended to study the following areas: 1. The biochemical group translocation transport mechanisms described for inosine and hypoxanthine uptake will be further analyzed using double isotope and other analytic techniques not previously applied. 2. The classical non-group translocation transport mechanisms that appear to coexist in many cells will be studied along with the above enzyme-mediated pathways in order to elucidate the role of each mechanism. 3. Specific mutants with altered uptake and purine utilization previously isolated in this laboratory and elsewhere will be employed to facilitate the proposed studies. 4. Cell growth, membrane isolation, transport assay, enzyme purification and surface topology (by iodination and serology) will be the principal methods employed.

458. LYMPHOID CELL PURINE NUCLEOSIDE PHOSPHORYLASE

Catsimpoolas, N., Griffith, A. L., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

The objective of this project is to study the enzyme purine nucleoside phosphorylase from lymphoid cells and other human cells in an attempt to establish the relationship between enzymatic deficiency and immunodeficiency. The presence and the levels of the enzyme in separated T and B lymphocytes and monocytes from peripheral blood will be determined, as will enzyme changes that may accompany stimulation of the above cells by mitogens and antigens. The enzyme will be purified from separated T and B lymphocytes, monocytes, blasts and macrophages and will be analyzed by two-dimensional gel electrophoresis to determine the state of subunit modification. The effect of subunit modification on the kinetic and catalytic properties of the enzyme will be assessed by isoelectric separation of the subunits of the erythrocytic enzyme (in the presence of 4 M urea) and reconstruction of homotrimers composed of only one type of subunit.

459. NORMAL AND DERANGED PURINE INTERMEDIARY METABOLISM (HUMAN, MONKEYS, RATS)

Balis, M. E., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

460. PHOTOCHEMISTRY OF PURINE N-OXIDES IN ONCOGENESIS

Parham, J. C., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

461. DAR - POSSIBLE INTERMEDIATE IN PURINE BIOSYNTHESIS (RAT, CHICKEN)

Baggott, J. E., University of Alabama in Birmingham, School of Medicine, Dept. of Medicine, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

This is part of a broader project. A summary of this subproject is not available.

462. METABOLIC STUDIES OF ONCOGENIC PURINE DERIVATIVES (RATS)

Stohrer, G., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

463. REGULATION OF PYRIMIDINE SYNTHESIS IN MAMMALIAN CELLS

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De novo pyrimidine biosynthesis in mammalian cells involves six enzymatic steps. The first three enzymes (carbamyl phosphate synthetase, aspartate transcarbamylase, and dihydroorotase) are associated with a single polypeptide of 220,000 daltons. The multimeric form of this polypeptide is called the CAD protein. At present little is known about the structure and regulation of this important and complex multifunctional protein.

In order to understand the structure and regulation of the CAD protein, the hamster protein will be cleaved by proteases into smaller fragments. Then biochemical techniques will be used to associate particular functions (such as enzyme active sites, substrate binding site, or aggregation center) with each

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fragment. Small fragments carrying one or a few functional domains (e.g., the allosteric regulator binding site) will be isolated in sufficient quantity for amino acid sequencing.

New and existent hamster mutants will help to characterize the functional domains of the CAD protein and to establish their order along the 220,000-dalton polypeptide chain. Such mutants also will be used to generate hamster/human somatic cell hybrids. Cytogenetic and biochemical analyses of such hybrids will permit the identification of the human chromosome carrying the CAD gene.

464. PYRIMIDINE SALVAGE PATHWAY INHIBITORS

Cha, S., Brown University, School of Medicine, Section of Biochemical Pharmacology, *87 Waterman St., Box 1883, Providence, Rhode Island,* 02912, United States of America

Recently this laboratory has discovered: 1) occurrence of a 5 prime-nucleotidase specific for orotidylic acid (OMP); and 2) Newly synthesized compounds, pyridimine acyclo nucleosides (1-(2'-hydroethoxymethyl) pyrimidines as potent inhibitors of uridine phosphorylase (both from normal and tumor cells) but without having any effect on thymidine phosphorylase, nucleoside kinases or 5 prime-nucleotidases.

Efforts will be made: 1) To elucidate the effects of uridine phosphorylase inhibitors on intact normal and tumor cells, and the interaction of these inhibitors with toxicities of other drugs such as 5-fluorouracil, 5-fluorodeoxyuridine, N-phosphonacetyl-L-aspartate (PALA) etc; 2) To search for more inhibitors of pyrimidine salvage enzymes; and 3) To determine profiles (i.e. activities) of all pyrimidine salvage enzymes in approximately 20 different types of cells (mostly malignant).

465. BIOCHEMICAL MECHANISMS OF PYRIMIDINE ALKYLATION

Sander, E. G., Rosenfield, C., Hedges, D. H., Texas A & M University, College Station Campus, School of Agriculture, Dept. of Biochemistry & Biophysics, *College Station, Texas,* 77843, United States of America

Studies will be continued on the allosteric regulation of dihydroorotase from a variety of sources. Special attention will be focused on the constitutive enzyme from C. oroticum. In addition, work will continue on the role of divalent cations in dihydroorotase and dihydrouracil amidohydrolase catalysis. We are especially interested in determining if the enzymes go via 'Zn-carbonyl' or 'Zn-hydroxide' mechanisms. The metabolism of the halogenated dihydrouracils will be studied.

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466. CONTROL OF PYRIMIDINE BIOSYNTHESIS IN MAMMALIAN CELLS

Evans, D. R., Grayson, D., Wayne State University, School of Medicine, Dept. of Biochemistry, *1014 Rivard, Detroit, Michigan,* 48207, United States of America

The objective of this research is to elucidate the control mechanisms involved in the regulation of de novo pyrimidine biosynthesis in mammalian cells. The first three enzymes of the pathway; carbamyl phosphate synthetase, aspartate transcarbamylase and dihydroorotase are associated with a single polypeptide chain. This large multifunctional protein, complex A, also exhibits allosteric transitions. The protein isolated from a mutant SV40 transformed hamster cell line, will be cleaved by controlled proteolysis to determine the number, function and arrangement of autonomously folded structural domains. These studies will employ chemical modification, peptide mapping, end group analysis and crosslinking experiments. The structure and interconversion of the oligomeric forms of the complex will be studied by gel filtration and ultracentrigation. Studied will focus on the unique functional properties such as channeling, reduction in transient time, coordinate effects and catalytic facilitation and the interrelationship of these properties to the allosteric control mechanisms. The kinetics of the system will be simulated by a computer model, to assess the extent to which compartmentation of intermediates can account for the functional properties of the complex. Using a combination of techniques, enzymatic assays, immunoprecipitation, gel electrophoresis and fluorecent microscopy, the activity, oligomeric form, intracellular concentration and location and the turnover of the molecule will be studied in cultured cells.

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467. GENETIC AND MOLECULAR ANALYSIS OF THE STRUCTURAL GENES FOR PYRIMIDINE BIOSYNTHETIC ENZYMES

Schedl, P., Princeton University, School of Natural Sciences, Dept. of Biology, *Box 430, Princeton, New Jersey,* 08540, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

468. CRYSTAL STRUCTURES OF PURINE AND PYRIMIDINE ANALOGS

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The objective of this investigation is to study the structural properties of purine and pyrimidine derivatives, and of enzymes that are involved in purine/pyrimidine metabolism. The main emphasis will be on investigations of the crystal structures of nucleosides, nucleotides and purines, and on crystallization and crystallographic studies of enzymes that interact with various purine derivatives. Particular attention will be given to alkaline-earth metal salts of nucleotides; purine analogues that are produced by alkylating agents that act on nucleic acids; and the enzymes adenosine deaminase and purine nucleoside phosphorylase. The crystallographic results will be used to examine the interactions of alkaline-earth metals with nucleotides; the effects that alkylation produces on the geometrical, conformational, tautomeric, hydrogen-bonding, and base-stacking properties of purines; and the structural factors that are involved in interactions of purine derivatives with enzymes. The ultimate aim is to identify specific structural properties that may contribute to the biological effects of purine and pyrimidine analogues.

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469. COENZYMES CONTAINING PURINE AND PYRIMIDINE ANALOGS

Parks, R. E., Agarwal, K. C., Stoeckler, J. D., Chen, S. F., Brown University, Graduate School, Division of Biology & Medicine, *University Hall, Box 1883, Providence, Rhode Island,* 02912, United States of America

This is an ongoing long-range study of the actions of purine antimetabolites on tumors and normal tissues. The studies involve several interrelated areas: 1) Adenosine deaminase (ADA) and its inhibition. The tight-binding inhibitor, deoxycoformycin (dCF) is under study in intact cells and whole animals. Evidence has been present indicating that the nucleoside transport system plays a rate-limiting role in the inactivation of intracellular ADA by dCF. Temperature dependence studies will be undertaken with both the on and off reactions with isolated enzyme and for further evaluation of the transport system. 2) 5'-methylthioadenosine phosphorylase (MTA-Pase), a target enzyme for drug development through studies with various 5'-methylthioadenosine (MTA) analogs, three types of potential agents have been identified: a) those that liberate a toxic adenine analog which is converted to analog nucleotides by APRT, b) those that liberate a toxic sugar-phosphate, e.g., 5-ribose-1-phosphate from 5'-iodoadenosine, c) competitive inhibitors of the enzyme, e.g., 5'-iodoformycin, 5'-chloroformycin; these analogs have Ki values of 1-5 times 10 to the minus 7th

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power M. Recent evidence indicates that certain of these compounds block mitogenesis in lymphocytes and produce promising inhibition in various tumor cells. 3) Analog nucleosides and blood platelet aggregation. Recently we have learned that the adenosine analogs, 2', 5'-dideoxyadenosine (ADA) and MTA, both block the inhibitory effects of adenosine on ADP-induced platelet aggregation. DDA overcomes the effects of prostaglandin, PGE1, whereas MTA does not. DDA inhibits platelet adenylate cyclase whereas MTA does not. Thus these analogs operate by different mechanisms. Since MTA is a naturally produced nucleoside, the possibility exists that it may play a role in the regulation of platelet behavior.

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470. METHYLATION OF RNA AND DNA IN PLANTS AND ANIMALS

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OBJECTIVE: Investigate the enzymatic methylation of purine and pyrimidine bases of RNA and DNA in plant tissues with the purpose of elucidating the possible relationship of such methylation to normal and abnormal regulatory processes in plant cells.

APPROACH: Determination of specific cellular location of RNA and DNA methylases in plants and development of assay method for plant methylases; determination of activity in vitro of DNA methylases and RNA methylase in cultured plant cancer cells produced by bacterial infection (crown gall tumors) and genetic imbalance (Kostoff hybrid tobacco tumors) and comparison with activity in normal and differentiating plant cells; study of relationship of nutritional states of crown gall cells to methylase activity; comparison of patterns of base-methylation with normal and cancer enzymes; partial purification and characterization of methylases from normal and cancer cells.

PROGRESS: Investigation of the role of transfer RNA in normal developmental processes will continue to increase our knowledge of how cells function and respond in a controlled way to their environment. Experiments concerned with investigation of transfer RNA metabolism in embryonic cells have been extended and research into the role of tRNA in juvenile hormone-induced development of specific organ functions in insects has been initiated. Previous experiments with crude preparations of tRNA methylases of avian liver have shown a higher enzyme activity in embryos than in mature birds when methyl-deficient avian tRNA, prepared by ethionine treatment, was utilized as substrate. Enzymes are currently being purified from avian livers and tested with ethionine-treated tRNA for detection of any specific differences in methylating ability related to developmental age. An analysis of tRNA's during juvenile hormone-induced developmental events in two different organs of adult cockroaches (Blaberus) has been initiated. A roach colony is being established and the patterns of major and minor species of a number of specific transfer RNA molecules are being determined by reversed phase chromatography on RPC-5 columns. tRNA from developing fat body and colleterial gland of adult female roaches will be analyzed. Juvenile hormone stimulates vitellogenin (yolk protein) production in adult fat body and synthesis of the components of the egg case (ootheca) by the colleterial gland of adult females.

V. CYCLIC NUCLEOSIDES AND NUCLEOTIDES IN NORMAL AND NEOPLASTIC CELLS

471. TRANSPORT VS ANTITUMOR EFFECTS OF NUCLEOSIDE ANALOGS

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A comprehensive evaluation of nucleoside transport by tumor and by normal proliferative cells is in progress. L1210 murine lymphocytic leukemia cells maintained as ascites in BD2F1 mice are used as the primary tumor model. Mature adsorptive and immature proliferative epithelial cells isolated from the villi and the crypts of the small intestine are used to study nucleoside transport by normal drug-sensitive cells. Multifaceted investigations with metabolically competent cells, metabolically altered (ATP depleted) cells, and membrane vesicles, as well as studies with nonmetabolizable analogs as 'model' substrates, are being used to determine the kinetic and physiological properties for the transport of natural nucleosides by tumor and by normal cells. The role of metabolism, especially phosphorylation, in the transport process is being considered. Competition studies between unlabeled nucleoside analogs and labeled natural nucleosides for transport by the same carrier are used to elucidate the structural specificity of the transport mechanism(s) in each cell type. These investigations will help us to understand whether or not the carrier-mediated uptake of nucleoside analogs contributes to the therapeutic response and selective action, or if it can be exploited to such ends by further structural modifications of the analogs.

472. RNA CHARACTERIZATION BY NUCLEOSIDE ANALYSIS

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This research proposes the development of a simple, selective and highly sensitive new research tool useful for investigations of RNA structure and function, and with which absolute composition analysis including identification and preparative isolation of previously uncharacterized modified ribonucleosides of any RNAs can be made at the nanogram level. Different RNAs will be quantitatively analyzed for their composition of major and modified nucleosides. The application of this chromatographic research tool will be most useful to investigations in biochemistry and molecular biology involving tRNA, mRNA, tumor and non-tumor viral RNAs. Very little is known about the influence of nucleoside modification on the function and regulatory activities of RNAs in viral transformation, protein synthesis, cell differentiation, neoplasia and suppression.

Our aims include development of a new research tool for nucleoside characterization that is simple, selective, and sensitive, with: a. optimization of enzymatic hydrolysis conditions of RNAs, b. utilization of boronate gel column chromatography for selective isolation and purification of RNA hydrolysates, c. modification and advancement of high-performance liquid chromatography (HPLC) to expand the range of modified nucleosides to be separated, identified, and quantitated, d. structural confirmation of known modified nucleosides and elucidation of structure of newly isolated modified nucleosides by mass spectrometry (MS), and if needed GC-MS, Ir, UV, NMR, etc., e. development of preparative isolation (mg amounts) of modified nucleosides in RNAs for structural characterization by NPLC-MS, GC-MS, IR,UV, and NMR. f. investigations on the structure and function of initiator and other tRNAs from Friend's leukemia cells undergoing differentiation.

473. CHEMOTHERAPEUTIC DEOXYRIBONUCLEOSIDES

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We propose to determine whether the structural gene for globin or the neighboring sequences are differently methylated depending whether the gene is expressed or not. For this purpose, DNA will be isolated from the active and inactive gene regions of uninduced and induced Friend cells using DNase II treatment and Mg ions precipitation. Both DNAs will be split with restriction enzymes which are specific for CG regions. The split products will be electrophoresed on agarose gels, transferred to Millipore strips, hybridized to radioactive globin cDNA. Since the cleavage by restriction endonucleases depends on the methylated state of cytosine residues, the autoradiographic profiles should reveal any changes regarding the methylated cytosine residues within or in the vicinity of the globin gene.

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DNA will be isolated from both the actively transcribed chromatin and inactive chromatin. DNA derived from the actively transcribed chromatin has proven to be a poor methyl acceptor, while DNA isolated from untranscribed chromatin accepts CH3 groups in vitro quite readily. We will attempt to establish that our finding is not an artifact due to the size of DNase II fragments, and propose to confirm these findings by extending them to other types.

We find that after DNase II treatment, DNA-methyltransferase activity is almost exclusively associated with the P2, or 'inactive', chromatin fraction. Minimal activity is associated with the S2, or 'active', chromatin fraction and this activity can be pelleted by high-speed centrifugation. These data indicate that the methyltransferase is bound to the nucleosomes. We will attempt to prove this assumption by showing 1) that DNase I treatment does not liberate any activity, 2) that the activity remains with the nucleosomes after micrococcal nuclease treatment.

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474. IDENTITY AND LOCALE OF ALKYLATED NUCLEOSIDES IN DNA

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The overall objective of this research proposal is to designate the identity and the regional sites of alkylated nucleosides in DNAs. An acutely sensitive and definitve mass spectrometric method (previously developed by the Principal Investigator) for the identification and localization of modified residues in viral DNAs will be applied to DNAs that have been altered by mutagenic and carcinogenic agents. The analytical method employs thermal and electron impact fragmentation of the intact DNA or DNA fragments and yields diagnostic ions which identify unusual purine or pyrimidine bases present in the DNA. Analysis of restriction endonuclease fragments, pyrimidine tracts, and other enzymatic degradation products from the DNAs will contribute to localizing the regions in the whole DNA molecule that contain the alkylated residues.

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475. NUCLEOSIDE, NUCLEOTIDE, AND POLYNUCLEOTIDE ANALOGS

Bergstrom, D. E., University of North Dakota, School of Arts & Sciences, Dept. of Chemistry, *University Station, Grand Forks, North Dakota,* 58201, United States of America

The preparation and reactions of organotransition metal derived complexes of nucleic acids and nucleic acid compounds are to be studied. The scope of the coupling reaction between chloropurine ribonucleosides and organonickel complexes will be investigated. Nucleic acids modified at the pyrimidine C-5 positions are to be synthesized via organopalladium intermediates. The modified polynucleotides are to be studied by 'H and 13C NMR spectroscopy.

A new synthetic route to fluoroolefins is under development. Fluoroolefins react with organopalladium and organonickel intermediates by pathways which may prove to be of exceptional synthetic versatility and utility.

C-5 substituted pyrimidine deoxyribonucleoside 5'-triphosphates are being examined as substrates for reverse transcriptase. Organopalladium chemistry may be used to synthesize analogs of the nuceloside antibiotic, tubercidin. In order to study the function of key enzymes utilizing ADP and ATP, a series of these analogs will be prepared and converted to their 5'-di- and tri-phosphates.

476. SYNTHESIS OF 2'-DEOXY C-NUCLEOSIDES

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The purpose of this proposal is two-fold: (1) to develop synthetic routes to 2'-deoxy C-nucleosides that are concise, employ readily available starting materials and result in intermediates that are conveniently transformable to a series of biologically interesting target compounds, and (2) to biologically evaluate these compounds with regard to their cytotoxic propensities in order to determine which compounds may exhibit promising antineoplastic activity and therefore should be explored further.

By applying synthetic procedures currently in use in other areas of organic chemistry, we believe that we will be able to develop versatile routes to 2'-deoxy C-nucleosides that will allow the realization of a series of derivatives of this class of compounds. The availability of a series of 2'-deoxy C-nucleosides will then allow a meaningful preliminary evaluation of their antineoplastic properties. The biological data accrued will, in turn, indicate which compounds warrant further study and what future, long-term directions our program should take.

The significance of this program is three-fold: (1) because of its versatility and potential for extrapolation, the methodology developed as part of this proposal will contribute significantly to the medicinal chemist's synthetic resources; (2) the potential antitumor activity associated with the products developed in this proposal may well result in significant contributions toward the search for new compounds for the treatment of neoplastic diseases; and (3) realization of the target compounds proposed in this program will allow a rational assessment of the structure-activity relationships exhibited by the pyrimidine-like 2'-deoxy C-nucleoside series of compounds.

477. MODEL ALKYLATED DECANUCLEOTIDE DNA HELICES

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A series of specifically modified, complementary deoxyribodecanucleotides (decamer) will be synthesized by the phosphotriester method. The modifications will include: (1) alkylated bases such as O6-ethylguanine, O4 ethylthymine and N3-methylcytosine, (2) alkylated phosphate groups (phosphotriesters), and (3) apurinic and apyrimidinic sites. These types of modifications are found in cellular nucleic acids after treatment with mutagenic and carcinogenic alkylating agents. The decamers will be capable of forming short DNA helices and duplexes in solution, with modificatons occurring in the central dimer region of the helix. Thus the helices will serve as models for DNA which has been altered by reaction with alkylating agents. The stability and thermodynamic properties of the modified helices will be studied by ultraviolet spectroscopic techniques. The overall mode of the base stacking in the modified helices will be observed by circular dichroism (CD) spectroscopic techniques. Proton and phosphorus nuclear magnetic resonance (NMR) spectroscopy will be used to study the conformation of the modified helices. Of particular interest will be the study of the perturbational effects of base and phosphate alkylation on the local conformation at the site of modification and to determine if these effects are propogated throughout the helix. The modified helices and duplexes will be tested for their ability to serve as model substrates for enzymes involved in DNA replication and repair including DNA polymerase, DNA ligase, DNA N-glycosylase and DNA apurinic endonuclease. The results of the physical studies will be correlated with the results of the biochemical studies.

478. CANCER CHEMOTHERAPY AND DEOXYNUCLEOTIDE METABOLISM

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The overall objectives of this proposal are to provide new information on (1) the development of deoxyribonucleoside analogs as new anticancer agents and (2) the logical use of combination chemotherapy in the therapy of neoplastic diseases. Initially, the properties of deoxyribonucleotide metabolizing enzymes in human tissues will be studied; enzymes such as ribonucleotide reductase, dUTP pyrophosphatase, N-glycosidase specific for uracil in DNA, dCMP deaminase and TMP kinase have so far not been well characterized and emphasis will be placed on evaluating the regulatory properties and the relationship between analog structure and enzyme activity. Previous experience in studying various human deoxyribonucleotide metabolizing enzymes will be used to develop methodology applicable to purification of these enzymes from a single source. Simultaneously, deoxyribonucleotide metabolism and regulation will be studied in order to gain knowledge useful in sensitizing or desensitizing human cells against deoxynucleoside analogs. Finally, the metabolism and mechanism of action of some active analogs will be examined with emphasis on two new promising antitumor analogs: 2'azido cytidine and ara-azido-cytidine.

479. COLONIC CYCLIC NUCLEOTIDE AND CARCINOGEN METABOLISM

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Our previous studies have indicated that (a) alterations in cyclic AMP(cAMP), cyclic GMP (cGMP) metabolism and/or cAMP-dependent protein kinase (PK) activity occur early after exposure of colonic mucosa to the carcinogens 1,2 dimethylhydrazine (DMH) (lower cAMP, lower PK activity ratio, higher soluble Type I PK), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (higher cGMP) or acetylaminobiphenyl (AAB) (higher cGMP); (b) alterations in cGMP are mediated by reactive derivatives of the carcinogens; (c) fatty acid dependent microsomal oxidation, linked in part to endogenous prostaglandin(PG) synthesis may be the preferred pathway for local drug and procarcinogen metabolism by colonic mucosa; (d) endogenous PG synthetic activity in colonic mucosa is an important local determinant of cAMP and cGMP; and (e) cAMP acts as a negative signal to cell proliferation in this tissue. The proposed studies are aimed at clarifying the potentially important relationships among fatty acid dependent procarcinogen oxidation, PG synthesis, and cyclic nucleotide metabolism, an understanding of which may suggest methods of pharmacologic and/or dietary modification of carcinogen and PGE metabolism. Accordingly, the goals of the proposed study are: 1) examine in cultured colonic explants of rat and human colons and in microsomal fractions fatty acid dependent oxidation of benzo(a)pyrene, DMH and azoxymethane and determine the extent to which potentially carcinogenic derivatives are formed by assessing the binding of these derivatives to nucleic acid; 2) examine the relationships among colonic mucosal fatty acid dependent procarcinogen oxidation, PG synthesis, cAMP and cGMP metabolism; 3) compare the effects of diets high and low in saturated versus unsaturated fatty acids or cholesterol on colonic PG synthesis, and on fatty acid versus NADPH induced procarcinogen oxidation; 4) examine the relationships between colonic mucosal PG synthesis and proliferative activity by assessing the effects of stimuli and inhibitors of PG synthesis on proliferation in colonic explants; 5) examine the roles of cAMP and cGMP in the control of colonic epithelial proliferative activity (DNA, RNA, and protein synthesis) and the possibility that cAMP induced inhibition of proliferation in this tissue is associated with phosphorylation of specific nuclear or membrane proteins.

480. THE ROLE OF NUCLEOTIDES IN MALIGNANT TRANSFORMATION

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The onset of DNA replication in mammalian cells is known to be regulated by cytoplasmic factors that act as positive regulatory signals. Acid-soluble nucleotides are likely to be used as intracellular signals because of their metabolic lability allowing their intracellular levels to fluctuate rapidly in response to conditions which affect growth. The objectives of the proposed project include the understanding of how nucleotides act in the regulation of DNA replication. Diadenosine 5', 5'''-P1,P4-tetraphosphate (Ap4A), a nucleotide formed in the back reaction of the first step of protein synthesis, was shown to be related to the proliferative activities of mammalian cells and to bind to DNA polymerase alpha with high substrate specificity. Recently we have demonstrated that the nuclear compartment pools of ATP and ADP can act as S phase regulators. DNA replication in isolated nuclei in vitro, was inhibited by high ATP levels and high ATP/ADP ratios. Studies of intact cells in vivo, were consistent with the in vitro results - although the total cellular pools of ATP and ATP/ADP ratios increase upon progression of mammalian cells through the G1 and into the S phase of their cycle, the nuclear compartment pools of adenine nucleotides and nuclear ATP/ADP ratios decrease upon entry into S phase of the cell cycle. Moreover, two nuclear enzymatic activities, nuclear ATPase and AMP deaminase, were shown to increase upon entry of mammalian cells into the S phase of their cycle and may be responsible for the cell cycle-related fluctuations in the nuclear pool sizes of adenine nucleotides. We propose experiments designed at establishing the regulatory role and site of action of Ap4A and ATP in relation to DNA replication. We plan to study the allosteric effects of Ap4A, ATP and ADP on enzymatic activities that are associated with proteins of the replication complex. Studies of regulatory mechanisms will be conducted on synchronized, intact and permeabilized cells as well as on isolated nuclei. Cell lines in which activation of host cell DNA replication is achieved by infection with tumor viruses will also be utilized.

481. REGULATION OF CYCLIC NUCLEOTIDE METABOLISM

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The guanine nucleotide-binding regulatory component of hormone-sensitive adenylate cyclase has been purified to homogeneity from rabbit liver and turkey erythrocytes. The biochemical properties of this protein will be assessed. Studies will emphasize (1) mechanisms of activation by guanine nucleotides and fluoride; (2) requirements for ADP-ribosylation by cholera toxin; and (3) mechanisms and consequences of interactions between the regulatory protein, the catalyst, and hormone receptors.

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482. ARRANGEMENT OF NUCLEOTIDE SEQUENCES OF HUMAN SATELLITE DNA'S

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OBJECTIVES: Eight satellite DNAs have been isolated from the human genome. It is not clear whether the satellite bands contain only repetitive sequences or whether they perhaps may contain unique sequences sequestered within repetitive sequences. The objective of this research is to determine the organization of base sequences in several human satellite DNAs.

APPROACH: Sequence specific restriction endonuclease will be used to probe the fine structure of human satellite DNA. Consider a satellite DNA arranged in a tandem array where the repeat unit is (baaaaa-------b) where both b and a represent a small sequence. When this satellite DNA is exposed to a restriction endonuclease one would expect the following consequences: (1) If each small sequence a and b contains a single restriction site in it, the whole array of repeats will be cut to the length of one unit a and units which separate a from b. (2) If the sequences of type b contain a restriction sequence, but those of type a do not, then long fragments will be generated which reflect the separation between b's; that is the long order repeat. If the site occurs more than once, fragments will be generated,

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the molecular weight of which will add up to the repeat length. (3) If neither a or b contains the restriction sequence the repeat unit is resistant to cleavage. Then the DNA segments containing multiple tandem copies of this unit are also resistant. The length of the cleaved fragments reflecting the length of the repeat can be characterized by gel electrophoresis. The frequency of the repeat can be calculated from the molecular weight of the starting material. Each defined fragment can be isolated and studied. Furthermore if the satellite DNA is susceptible to cleavage by other enzymes, it is possible to orient the various restriction sites within the repeat unit by comparing the products of digestion of the enzymes working separately and together on the DNA.

483. NUCLEOTIDE BIOSYNTHESIS AND DEGRADATION

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Extracts of human lymphocytes analyzed by high-performance liquid chromatography on an anion exchange column showed a major UV-absorbing peak which eluted after the nucleosides and prior to the major nucleotides. This material co-chromatographed with and had the spectral properties of ascorbic acid. Further evidence that the compound was ascorbic acid was obtained with chemical and enzymatic assays. Following its unequivocal identification, we determined the levels of the UV-absorbing compound in chronic lymphocytic leukemia lymphocytes. Normal lymphocytes had 39.6 plus or minus 18.8 n moles/10 to the 8th power cells (mean plus or minus S.D.). Lymphocytes from patients with chronic lymphocytic leukemia were found to have significantly higher levels (113 plus or minus 52 n moles/10 to the 8th power cells, p less than 0.001.

484. KINETICS OF ALKYLATION OF RNA COMPONENTS BY EPOXIDES

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Kinetic studies of reactions of the diastereomeric 7,8-diol 9,10-epoxides of benzo (a) pyrene (BAP) with nucleotides and nucleosides in aqueous solutions will be carried out. Kinetic results indicate that the BAP diol epoxides are very reactive toward 5'-guanosine monophosphate and initial product studies indicate substantial covalent binding of diol epoxide to the nucleotide. Additional product studies will be conducted to determine the nature and extent of binding. Similar kinetic and product studies of the reactions of diol epoxides with adenosine and adenosine monophosphate will be initiated.

485. CHARACTERIZATION OF RNA/DNA IN ONCOGENIC SYSTEMS

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The functional significance of various methylated nucleosides present in the nucleic acids of tumor cells and of DNA- and RNA-tumor virsues are being investigated by various immunochemical techniques. This approach employs antibodies specific for various methylated nucleosides which include 7-methylguanosine (m7G),N2,N2-dimethylguanosine (m2G), N6-methyladenosine (m6A), and 5-methylcytidine (m5C). Upon production, characterization, and purification, antibodies will be employed to isolate and characterize nucleic acid possessing the corresponding methylated nucleosides. Nucleic acids of particular interest include Adenovirus DNA and mRNAs, Rous Sarcoma Virus RNA and various bateriophages such as fd.

The interaction between a specific anti-methylated nucleoside antibody and various nucleic acids provides unique immunochemical approaches for the (i) detection and (ii) quantitation of methylated constituents present in nucleic acids. Additionally, electron micrographs of nucleic acid:antibody complexes can be utilized (iii) to map the position of methylated nucleosides within a nucleic acid while immobilized antibodies can be employed (iv) to isolate hapten-containing (deoxy)oligonucleotides and nucleic acids. Last, the inhibition of specific enzyme activities associated with the metabolism of nucleic acids by the appropriate nucleic acid:antibody complexes will provide information regarding the (v) functional role(s) of the methylated nucleosides being examined.

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486. NMR PROBES OF ENZYMES AND NUCLEIC ACIDS

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Several new areas of development and application of the dynamic nmr (nuclear magnetic resonance) probe of the active site of enzymes will be explored. We hope to provide a novel experimental methods for probing the struture of the transition state enzyme complex by studying the nmr spectra of transition state analogs as they reversibly bind to the enzyme. By also applying these techniques to substrates and intermediates stabilized at low temperatures, it should be possible to achieve a detailed understanding of the enzymatic mechanism of action. In addition an nmr study of covalent substrate intermediates will be presented. This should provide answers as to whether a covalent intermediate does, in fact, exist and, most importantly, define the amino acid residue which covalently links to the substrate.

These nmr techniques will be applied to the study of ribonuclease A, alpha-chymotrypsin, 3',5'-cyclic-AMP dependent protein kinase, phosphoglycerate mutase and UDP, Galactose epimerase. However, broad application of these ideas to other enzymes are anticipated as well.

In addition, our recently proposed 31P chemical shift-torsional angle hypothesis will provide an important new probe of nucleic acids structure. We hope to study the conformational flexibility about the phosphate ester backbone of oligonucleotides and polynucleotides (t-RNA, 5S RNa) by 31P nmr spectroscopy. We plan to probe with this technique subtle polynucleic acid, drug, protein, and carcinogen interactions.

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487. STRUCTURE AND FUNCTION OF FLUORINATED NUCLEIC ACIDS

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The objectives of the proposed research are: (1) To determine the detailed physiochemical and conformational properties of nucleic acids and their constituents in which hydrogen atoms have been selectively replaced with fluorine atoms; (2) to determine the translation properties (using an in vitro mammalian system) of model nucleic acids containing fluorinated bases to determine their potential for miscoding; and (3) to evaluate fluorinated nucleic acid constituents as nuclear magnetic resonance (NMR) probes in studying the structure and function of macromolecules in vitro. The methods include both chemical and enzymatic synthesis of the nucleotides, oligo-, poly-, and copolynucleotides to be used as model nucleic acids. These will be analyzed by ultraviolet (UV), circular dichroism (CD), and NMR spectroscopies to determine their solution properties, and selected constituents will be analyzed by X-ray diffraction to determine their solid state conformation. A mammalian in vitro protein-synthesizing system will be used to determine the extent and conditions of miscoding by synthetic fluorinated-mRNA.

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488. STRUCTURE AND FUNCTION OF NUCLEIC ACIDS

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Oligodeoxynucleotides of specific base sequence will be synthesized to provide examples of structural elements in DNA. The stability of perfect helices and of mismatched helices will be measured as a function of temperature, salt concentration and solvent polarity. The effect of covalently and noncovalently bound mutagens will be assessed. The structures of DNA-mutagen complexes will be determined using circular dichroism, temperature-jump kinetics, nuclear magnetic resonance and other physical properties.

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VI. BROAD STUDIES ON NUCLEIC ACID BIOCHEMISTRY

489. BASIC NUCLEIC ACID STRUCTURES - FORM AND INTERACTION

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Investigations into the conformation and stability of basic nucleic acid structures and their interactions with other biologically important molecules are proposed. Knowledge of the properties of these basic structures is a key to our understanding the function of nucleic acids in the synthesis of proteins, the operation of the genetic apparatus, the regulation of cellular processes, and the mode of action of chemical agents that cause mutation and cancer.

Enzymes will be used to synthesize especially interesting oligomeric components of larger nucleic acids. The purification of these oligomers will be greatly aided by a high pressure liquid chromatographic (HPLC) method we have developed. Nuclear magnetic resonance (nmr) experiments will be employed to investigate the structural details of these molecules in aqueous solution. These experiments will be correlated with studies of the absorption of ultraviolet light as the ordered nucleic acid structures are disrupted by increasing the temperature. This information bears upon the stability of the natural counterparts of these synthetic nucleic acids.

Further studies on the interactions of synthetic oligonucleotides with drugs, mutagens, and metal ions will lead to interference about the molecular basis of mutagenesis and carcinogenesis as well as providing an approach to rational drug design.

490. MOLECULAR STRUCTURE OF NUCLEIC ACIDS AND PROTEINS

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The object of this research program is to carry out biophysical and biochemical studies on the nucleic acids in order to provide insight concerning the mechanism of their action in biological systems. A central part of this program is to use the information on the recently determined three-dimensional structure of transfer RNA in order to understand more fully its mode of action in biological systems. One way of obtaining this information is to determine the three-dimensional structure of proteins which interact with transfer RNA. X-ray diffraction techniques will be used to solve the structure of these macromolecules. In addition, attempts will be made to co-crystallize transfer RNA with proteins with which it interacts. The molecular structure of these complexes will be determined and they should provide significant insight into the manner in which proteins recognize nucleic acids. Crystallographic studies will also be carried out on other nucleic acid components, including oligonucleotides, in order to study their natural conformation as well as the manner in which their conformation is modified by interaction with mutagens or carcinogens. This includes the structure of DNA fragment of defined sequence.

Another part of this research project is related to the mechanism of action of nucleic acids in protein synthesis. Studies will be carried out on specificity of tRNA aminoacyl synthetases as well as the manner in which tRNA is involved in codon-anticodon interactions.

BIBLIOGRAPHIC REFERENCES: Initiator tRNAs have a unique anticodon loop conformation by Paul Wrede, Nancy H. Woo and Alexander Rich, Proceedings of the National Academy of Sciences USA 76, 3289-3293 (1979). Structure and Function of a Gene Product: Yeast Phenylalanine Transfer RNA by Alexander Rich in Specific Eukaryotic Genes, Ed. by J. Engberg, H. Klenow and V. Leick, Munksgaard, Copenhagen (1979), pp. 472-487.

491. COENZYMES AND NUCLEIC ACIDS METABOLISM

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Our continued research efforts will concentrate on metabolic studies and chemotherapy of human tumors maintained in the athymic mice and in culture and immunology of the athymic mice. Investigations to determine whether the athymic mouse can serve as a model for chemotherapeutic agents for human cancers will be continued. Detailed investigations on the use of antibodies as a potential cytotoxic agent will be continued. The pareparation of conjugates using monoclonal antibodies and cytotoxic agents will be carried out. Their characteristics, chemically and biologically, will be investigated. Chemical properties of human tumors grown in the athymic mouse will be studied. The relation of growth of human tumors in the athymic mouse as related to metabolic parameters will also be investigated. The biochemical changes produced by certain factors in the growth medium of human tumor cells will be delineated.

We have recently found a virulent form of mouse lymphoma in athymic mice. We shall carry out studies to determine the factors that induce this lymphoma. The biochemical parameters of this induction, as well as the chemical changes that take place in the lymphoma itself, will be studied. These lymphomas will be characterized both morphogically and biochemically.

BIBLIOGRAPHIC REFERENCES: J. Lipsick, G. Beattie, A.G. Osler and N.O. Kaplan. Passive Transfer of Lymphocytes from Diabetic Man to Athymic Mouse. Lancet, 1, 1290 (1979). L.J. Arnold, Jr., A. Dagan, J. Gutheil and N.O. Kaplan. The Anti-Neoplastic Activity of Poly-l-Lysine with Some Ascites Tumor Cells. Proc. Natl. Acad. Sci. USA, 76, 3246-3250 (1979).

492. NUCLEIC ACID BIOCHEMISTRY OF CELLS

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The nucleic acid investigations are targeted towards the effects of deleterious environmental pollutants associated with the chemistry and use of fossil fuels. The project has initiated studies on cell lines derived in vitro from tracheal epithelial explants previously exposed in vivo to chemical carcinogens. The objective is to investigate changes in RNA biochemical pathways in tracheal epithelial cells as they progress from a clearly nonmalignant to an altered and finally to a malignant state during subculture in vitro. Events marking the evolution of neoplasia (alterations in growth capacity, morphological transformations, and acquisition of tumorigenicity) will undoubtedly correlate with transitions in the regulation of transcription of these cells. Previous experiments will continue to be directed toward evaluating molecular mechanisms of RNA transcription, processing and degradation associated with chemical toxicity.

493. NUCLEIC ACID HYBRIDIZATION CORE FACILITY

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This is part of a broader project. A summary of this subproject is not available.

494. PROTEIN AND NUCLEIC ACID STRUCTURE SECTION (MAMMALS)

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This is part of a broader project. A summary of this subproject is not available.

495. CHARACTERIZATION OF NUCLEIC ACIDS

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This is part of a broader project. A summary of this subproject is not available.

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